VOLATILE-MEDIATED ARTHROPOD-FUNGUS INTERACTIONS

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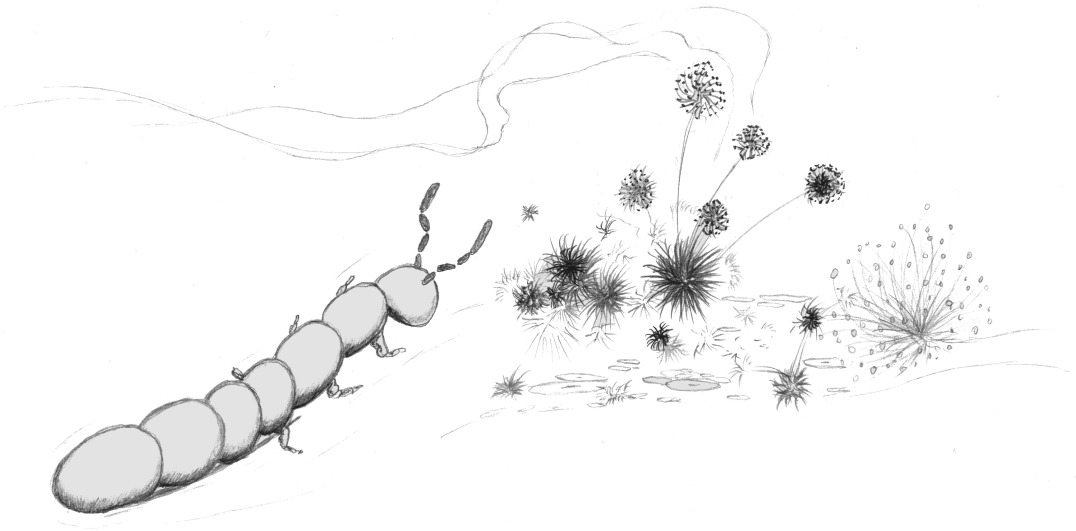
'Man is Nature becoming aware of itself'

(Jacques Élisée Reclus)



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SUMMARY

Fungi are considered as main decomposers in terrestrial ecosystems and, due to their diverse lifestyles, engaged in a plethora of interactions with soil invertebrates. Determining the underlying mechanisms that regulate interactions with invertebrates is of major importance for understanding the dynamics of soil fungal and animal communities and assessing the impact of these interactions on ecosystem processes and functions. The outcome of invertebrate-fungus interactions has been assumed to depend strongly on fungal chemical properties, viz. the production of non-volatile and volatile secondary metabolites. Soil invertebrates are known to use fungal-derived volatiles as cues for the location of suitable habitats, feeding, and oviposition sites. However, especially with regard to springtails (Collembola) and woodlice (Isopoda), a direct link between the dynamics in fungal volatile emissions and the behaviours underlying food location and selection of these important decomposers is still missing. The aim of the present PhD project was to investigate this aspect in more detail by combining analyses of fungal volatile profiles and behavioural responses of animals by continuous video observation and by focussing on different behavioural components of the food selection process separately.

The first main intent was to find out whether a certain group of volatiles, namely oxylipins, are used as cues by isopods and Collembola during foraging. Derived from the fact that the constitutive and wound-activated emission is a conserved mechanism in higher fungi I assume that oxylipin volatiles are ubiquitous in soil habitats. Since oxylipin volatiles are well known to play significant roles as infochemicals in plant-insect interactions I hypothesised that oxylipin volatiles are of similar importance in mediating interactions between fungi and soil invertebrates and function as food-finding cues for Collembola (*F. candida, S. curviseta, H. nitidus*) and isopods (*O. asellus*). Unexpectedly, wound-activated increases in oxylipin emissions did not increase the food-finding efficiency of these animals, however, independent of the wounding treatment, isopods were found to be attracted to *C. globosum* fungal colonies. Moreover, the common fungal oxylipin volatile 3-octanone arrested isopods in close proximity to the volatile source. This provides very first evidence of a role of fungal volatiles in affecting the foraging behaviour of isopods and indicates that isopods use volatiles as information to locate fungal food from a distance. Furthermore, upon direct contact with fungal colonies, fungal tissue wounding increased the acceptance of *C. globosum* colonies as food source by Collembola and, most interestingly, the mere presence of

3-octanone elicited test-biting behaviour in these animals, a here newly-observed component of the Collembola foraging behaviour. These findings strongly suggest that oxylipin volatiles, at least 3-octanone, in fact influence the foraging behaviour of both Collembola and isopods by acting as attractants, arrestants, and phagostimulants.

The second main intent was to investigate whether Collembola (*F. candida*) are able to differentiate between fungi of varying suitability by means of volatiles. Therefore, I observed behavioural responses of Collembola to different yeasts and filamentous fungi, determined fitness consequences of the respective fungal diets, and analysed fungal volatile profiles. In line with expectations, volatile-mediated responses of Collembola were largely reflected in the acceptance of fungi as food source and finally in the fitness of the animals (growth and reproduction), indicating that the use of volatile cues is important for Collembola to optimise their fitness. The observed higher attractivity and acceptance of yeasts and the filamentous fungus *A. nidulans* can most likely be attributed to the presence of 3-methyl-1-butanol within an otherwise volatile-poor background. Interestingly, Collembola were deterred by the volatile bouquet of the filamentous fungus *P. expansum*, did not accept this fungus as food source and had the lowest fitness increase. Responses to the *Penicillium* characteristic terpenoid geosmin indicate that this compound may contribute to the repellent effect. Possibly, some fungi produce such repellent compounds to fend off predators. These results clearly show that Collembola discriminatingly use fungal-derived volatile cues to make adaptive foraging decisions. Besides chemical properties, differences in physical properties (unicellular or hyphal growth) may influence the accessibility and consequently the acceptance of fungal food sources by Collembola, however, this needs to be tested in future studies.

Overall, this thesis provides further evidence of a significant role of fungal chemical properties, volatiles in particular, in influencing the outcome of fungus-invertebrate interactions. Future studies should focus on investigating whether the here observed behavioural responses of isopods and Collembola actually reflect responses exhibited in their natural habitat.

CHAPTER 1

A REVIEW OF THE ROLE OF FUNGAL VOLATILES AS INFOCHEMICALS IN INVERTEBRATE-FUNGUS INTERACTIONS

1.1 INTRODUCTION

Fungal and animal communities are interconnected via a multitude of interspecific interactions. Fungi are important mutualists, prey, predators and competitors of enormously species rich invertebrate communities, comprising insects, mites, isopods, nematodes, and molluscs (Figure 1.1). Therefore, obtaining a better understanding of the factors regulating interactions with invertebrates is crucial for predicting the composition of fungal communities and the involvement of these inherently dynamic interaction processes in ecosystem functioning (Crowther *et al.* 2012; 2011a;b; 2013).

Like in plant-animal interactions (Schoonhoven *et al.* 2005), the establishment of short- or long-term intimate fungus-invertebrate contacts as well as the disintegration of such contacts are likely driven by fungal chemistry (Gloer 1995, Rohlfs 2015). The 'decision' of invertebrates to pick up, feed on or avoid a fungus may thus be based on the perception and neurophysiological integration of fungus-borne chemical information. Compared to plant-animal interactions, however, we have only fragmentary knowledge about the role of such fungal infochemicals in regulating interactions with invertebrates.

Because of their physicochemical properties infochemicals can be roughly classified as non-volatile and volatile compounds. Those fungal metabolites that tend to vaporize easily, i.e. to transit from liquid to gas phase in a temperature range in which invertebrates are active, have the potential to act as infochemicals. The so far ~250 identified fungal volatile metabolites (Morath *et al.* 2012) originate from different biosynthetic pathways based on amino acid, fatty acid or mevalonate precursors (Korpi *et al.* 2009). Therefore, fungi often emit complex mixtures of volatiles comprising alcohols, organic acids, alkenes, aldehydes, acetates, sulphides, and terpenoid compounds. Invertebrates, in particular insects, have evolved a high

diversity of receptors to perceive many of these metabolites (Münch and Galizia 2016). Fungal volatile metabolites may thus 'inform' invertebrates about the presence and properties of fungi without direct contact and trigger fitness-relevant decision-making, e.g. optimal foraging and mutualist choice, avoidance of fungal pathogens, etc.

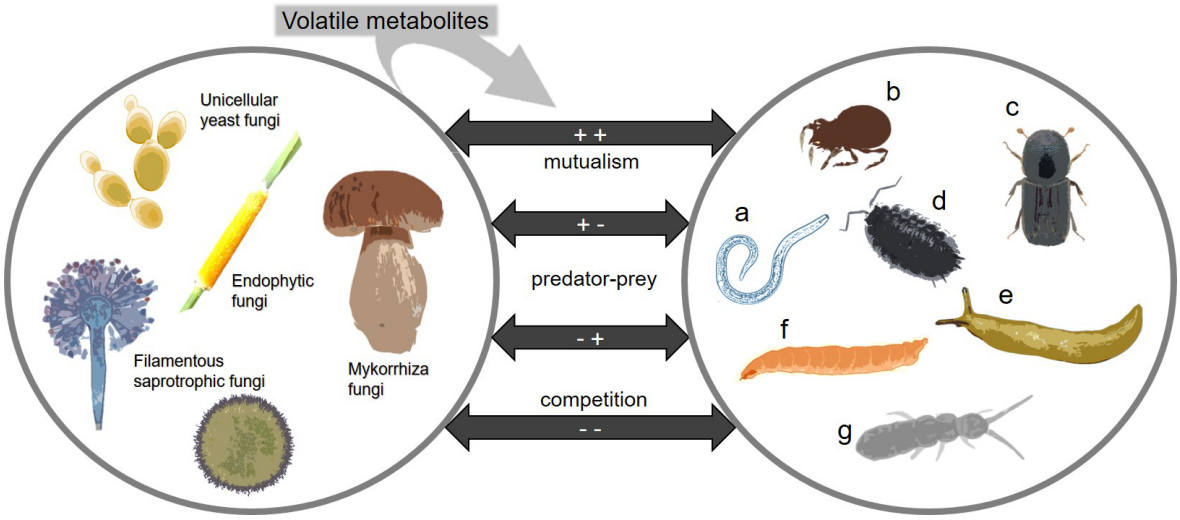

Figure 1.1: *Interaction types categorised by the effect potentially mediated by volatile metabolites. (a) nematode, (b) mite, (c) beetle, (d) isopod, (e) mollusc, (f) fruit fly larva, (g) springtail.*

The aim of this review is to compile and critically evaluate the current evidence of fungal volatile metabolites as infochemicals for invertebrates. The focus is mainly on bipartite invertebrate-fungus interactions; however, I also elaborate on the potential role of fungal volatiles as infochemicals in regulating the strength of multispecies interactions.

1.2 FUNGI AS DIET

Several studies have demonstrated that fungal-derived volatiles and/or fungal authentic compounds affect the foraging behaviour of fungivorous springtails, beetles, flies, woodlice, earthworms, mites, and slugs (Table 1.1). Springtails (Collembola) and mites (Acari) are assumed to use olfactory cues in the recognition and discrimination of fungal food sources (Bengtsson *et al.* 1988; 1991, Brückner *et al.* 2018, Hedlund *et al.* 1995, Sadaka-Laulan *et al.* 1998, Staaden *et al.* 2011, Vanhaelen *et al.* 1980). Bengtsson *et al.* (1991) have demonstrated that springtails are able to adjust their behaviour in the presence of different fungal authentic volatile compounds (Table 1.1), indicating behavioural plasticity. In olfactometer

experiments groups of euedaphic (soil living) springtails (*Onichiurus* spp.) were attracted to a number of different fungal species, however, the degree of attraction varied between fungal species and in relation to species- and substrate-specific qualitative and quantitative differences in volatile profiles (Bengtsson *et al.* 1988; 1991, Sadaka-Laulan *et al.* 1998). Here it is important to mention that, in general, when testing groups instead of individual animals it remains unclear if behavioural responses are affected by fungal-derived volatiles or additionally influenced by the odour of conspecifics. Sensory detection of and volatile-mediated responses to fungi also differ between springtail species as it has been shown for sympatric *O. cincta* and *T. flavescens* and it is suggested that these differences contribute to differences in food selection by springtails (Hedlund *et al.* 1995). According to the mixed diet hypothesis springtails benefit from using different food sources for covering nutritional requirements and are considered as food generalists (e.g. Scheu and Simmerling 2004). Although there are species-specific preferences for certain fungi, the finding that springtails are attracted to a wide range of fungi and to rather common widespread authentic volatile compounds (e.g. CO2, alcohols) (Table 1.1) (Bengtsson *et al.* 1991) supports this hypothesis. A more detailed observation of individual springtails by continuous video observation revealed that *F. candida* springtails are able to adjust their behaviour in the presence of food by switching from random non-directed to directed movement towards a non-fungal volatile source, but only from short distance (2.5 cm) (Auclerc *et al.* 2010). In contrast to the short-distance response of *F. candida* it has been shown that *O. armatus* springtails were attracted from larger distances (40 cm) to a fungal food source and that movement and dispersal was even more strongly affected by fungal volatiles than by population density (Bengtsson *et al.* 1994). Sawahata *et al.* (2008) have demonstrated that aggregation of springtails on the palatable basidiomycete *T. matsutake* was weaker compared to other fungi and caused by a repellent effect of the fungal volatiles 1-octen-3-ol and methyl cinnamate, suggesting a role of volatiles in the direct defense of fungi against fungivory. Furthermore, volatile-mediated avoidance of certain fungi is assumed to be associated with fungal toxicity (Sadaka-Laulan *et al.* 1998) and was explicitly investigated by Staaden *et al.* (2011) who have shown that *F. candida*, *H. nitidus*, and *S. furcifera* springtails preferred a mutant strain of the ascomycete *A. nidulans*, deficient in the production of secondary metabolites including the toxic compound sterigmatocystin, over a sterigmatocystin producing wild type strain. This finding strongly suggests that springtails are able to detect and avoid unpalatable toxic fungi from a distance by employing fungal-derived volatiles. However, discrepancies between volatile-mediated attraction and acceptance of a fungus as food resource (Sadaka-Laulan *et al.* 1998)

indicate that springtails use additional cues for the evaluation after arrival at a potential fungal food source. The additional use of other cues during the contact phase was also suggested to be relevant for successful foraging of *O. asellus* woodlice (Isopoda) as the observed preference for mouldy food was only significant after physical contact to a potential food source which indicates that olfactory cues could not be detected or do not play a role during the non-contact searching phase (Zidar *et al.* 2003). In contrast to *O. asellus*, *P. scaber* woodlice were attracted to litter and alpha-cellulose inoculated with microorganisms (undefined) and are assumed to employ volatiles which derive from the microbial breakdown of cellulose for the location of suitable feeding sites (Zimmer *et al.* 1996). As it has been shown for example for the characteristic mushroom volatile 1-octen-3-ol, fungivorous animals can respond very differently to the same volatile compound. Whereas *Proisotoma minuta* springtails, *M. halterata* flies, and *A. columbianus* slugs were repelled/deterred by 1-octen-3-ol (Pfeil and Mumma 1993, Sawahata *et al.* 2008, Wood *et al.* 2001) it arrested *O. armatus* springtails (Bengtsson *et al.* 1991), and attracted fungivorous *C. moschata* beetles (Tabata *et al.* 2011) and *Scheloribates* mites (Brückner *et al.* 2018). This indicates that the perception and processing of fungal olfactory signals could have specifically evolved in fungus-invertebrate interactions depending on the characteristics of invertebrate nutritional requirements and fungal chemistry.

From the studies described above it can be assumed that, at least, for some soil-living fungivorous arthropods (springtails, woodlice) fungal-derived olfactory cues act as semiochemicals under laboratory conditions, however, one important question arises: Do fungal volatiles also influence the foraging behaviour of invertebrates under more natural conditions with consideration of the complex obstacle-rich structure of soil systems? Taking this aspect into account, Zirbes *et al.* (2011) investigated the foraging behaviour of *E. fetida* (Annelida) in response to volatiles from filtrates and authentic compounds of the ascomycete *G. candidum* under semi-natural conditions by using below-ground olfactometers filled with moist compost soil. Groups of earthworms were attracted to fungal volatiles horizontally as well as vertically up to a distance of 105 cm, indicating that fungal volatiles, in fact, can act as infochemicals from a significant distance despite the obstacle-rich structure of a soil environment (Zirbes *et al.* 2011).

1.3 FUNGI AS HOSTS

Living together with host fungi brings a twofold advantage; fungal fruiting bodies provide a permanent food resource and additionally serve as shelter for both larvae and adults whereby foraging costs are eliminated. In turn, some arthropods are suggested to act as vectors of fungal spores or gametes and specific fungal volatile compounds are assumed to have evolved into 'pollinator'-attracting signals (Cloonan *et al.* 2016a, Schiestl *et al.* 2006, Steinebrunner *et al.* 2008a).

Xylobiont (bracket fungi) and deadwood inhabiting fungi form microhabitats that provide optimal living conditions for associated animals. Many beetle species, in particular bark beetles, are known to use fungi as hosts and often have species-specific symbiotic relationships with their fungal associates (Vega and Blackwell 2005). Guevara *et al.* (2000a;b) have demonstrated that specialist ciid beetles use fungal species-specific volatile cues to discriminate between host and non-host fungi and, moreover, that the use of age-related characteristics in the volatile bouquet of the host fungus (*C. versicolor*) provides a mechanism for resource partitioning by *O. glabriculus* and *C. boleti*, both strongly associated with this fungus. In line with this, the specialist fungivorous beetle species *B. reticulatus* also relies on age-specific characteristics in volatile bouquets of their xylobiont host fungus, in particular the presence of the ubiquitous eight-carbon oxylipin volatile 3-octanone, to differentiate between young and fresh fruiting bodies and the preferred partially dead successional stage of this fungus (Holighaus *et al.* 2014). 3-octanone and other oxylipin volatiles, e.g. 1-octen-3-ol, 3-octanol, and 1-octanol, have been demonstrated to attract different bark beetle as well as some grain beetle species, suggesting that volatile oxylipins play an important role as infochemicals and key attraction factors in the host selection behaviour (Drilling and Dettner 2009, Fäldt *et al.* 1999, Pierce *et al.* 1991, Thakeow *et al.* 2008). Besides beetles, saproxylic bed bugs (*A. obtectus*) and wood wasps (*S. noctilio*) have been demonstrated to use fungal volatile cues for the location of their host fungi (Koban *et al.* 2016, Sarvary *et al.* 2016).

Volatiles also play a significant role in the strong mutualistic interaction between endophytically living *Epichloë* fungi and *Botanophila* flies that use different *Epichloë* species for feeding and oviposition and in return act as gamete vectors. Field bioassays by Schiestl *et al.* (2006) and Steinebrunner *et al.* (2008b) revealed that *Epichloë* characteristic volatile compounds, namely Chokol K and methyl (Z)-3-methyldodec-2-enoate, function as key attractants specifically for female *Botanophila* flies and direct them to the fungal stomata. Interestingly, Chokol K additionally inhibits the growth of mycoparasites and plant pathogenic

fungi and it is suggested that the function of this compound as *Botanophila* attractant has evolved from its original function as fungal defense (Steinebrunner *et al.* 2008a).

1.4 FUNGAL NICHE CONSTRUCTION

Some fungi act as niche constructors by degrading and modifying plant substrates like deadwood and fruits and create suitable microhabitats for subsequent insect colonisers. On the one hand, in respective systems, interactions between insects and fungi often have a mutualistic character; whereas fungi are used as food source and provide optimal conditions for insect larval development, insects often benefit fungi by vectoring fungal spores or cells to new substrates (e.g. Ganter 1988, Gilbert 1980, Madden *et al.* 2018, Starmer *et al.* 1988). In this way insects can strongly affect the density and diversity of the fungal community and simultaneously benefit themselves by establishing their own niches (Buser *et al.* 2014, Stamps *et al.* 2012). Volatiles that were frequently demonstrated to act as attractants are mainly ubiquitous compounds - alcohols, acids, acetates, and aldehydes - produced by fungi during fermentation of sugar rich substrates (Table 1.1). The significance of fungal volatile cues in mediating attraction to fungal-colonised plant substrates was demonstrated with respect to fruit flies, moths, and beetles.

Although *Drosophila* fruit flies are well known to rely on fungal volatile cues for the long-distance location of fungal-colonised fruit substrates that serve as food and oviposition sites (Becher *et al.* 2010; 2012), a study by Palanca *et al.* (2013) suggests that the *Drosophila*-yeast mutualism may have evolved independently of fermentation since adult flies were attracted to both weakly or non-fermenting yeast species and fermenting species. Different *Drosophila* species exhibit volatile-mediated preferences for different yeasts (Buser *et al.* 2014, Dobzhansky *et al.* 1956, Scheidler *et al.* 2015) that are reflected in fitness benefits for both the fruit flies and the yeasts (Buser *et al.* 2014). Scheidler *et al.* (2015) have demonstrated that yeast species-specific behavioural responses of *D. melanogaster* and *D. suzukii* correlated with olfactory responses (electroantennography), indicating that these insects are able to detect species-specific chemical characteristics in yeast volatile profiles. This suggests a strong coadaptation between certain *Drosophila* and yeasts species (Scheidler *et al.* 2015) which is encouraged by volatile signalling.

Similar to fruit flies, adult grapevine-, apple-, and codling moths (*L. botrana, E. postvittana, C. pomonella*) use yeast-derived volatile cues for the long-distance location of yeast-colonised

fermented fruit substrates that are used as food and oviposition sites (Tasin *et al.* 2011, Witzgall *et al.* 2012). It is assumed that the presence of mutualist and antagonist fungi affects the quality of the host plant as larval food. Contrary to the preference-performance hypothesis, oviposition choices made by adult moths were less reflected in the survival of their offspring. While *E. postvittana* and *L. botrana* larvae benefited from the presence of the grey mould fungus *B. cinerea* and were attracted by volatiles of this fungus (Mondy *et al.* 1998, Rizvi *et al.* 2016), adult female moths were repelled and avoided oviposition on *B. cinerea*-infected berries (Tasin *et al.* 2011; 2012). Rizvi *et al.* (2016) suggested that the discrepancy between larval and adult behavioural responses may be due to differences in host requirements and/or feeding preferences.

Sap beetle species (Nitidulidae) prefer yeast-colonised fruit substrates as food source and oviposition site over uncolonised fruits (Blackmer and Phelan 1991, Nout and Bartelt 1998). It has been demonstrated that these beetles were attracted by different yeast species and blends of volatiles that were previously identified from attractive yeasts (Lin and Phelan 1991, Nout and Bartelt 1998, Phelan and Lin 1991). By means of subtractive and additive bioassays Lin and Phelan (1991) found out that attraction can mainly be assigned to certain volatile compounds - acetaldehyde, ethyl acetate, and 2-methylpropanol - single compounds, however, were less attractive than the respective three-compound blend (Table 1.1), indicating that the composition of yeast volatile profiles is more important for the detection of suitable microhabitats by sap beetles than the mere presence of single compounds. Moreover, Lin and Phelan (1992) have shown that sap beetles were attracted to volatiles derived from the insect-dependent yeast *C. fagacearum* but not to yeast species that are primarily dispersed by wind or water and concluded that the volatile bouquet of *C. fagacearum* is adapted for attracting sap beetles and/or other insect vectors. Similarly, different species of fungus-growing ambrosia beetles were also demonstrated to rely on volatile cues for locating and selecting their respective fungal symbionts from a distance (Hulcr *et al.* 2011). These beetles create their own niche by vectoring fungal spores to dead wood substrates and actively cultivating the so-called 'fungal gardens'. The presence of morphological adaptations (mycangia) for the transport of fungal spores (Vega and Blackwell 2005) and the finding that the strongest attraction of ambrosia beetles is elicited by the volatile bouquets of their respective fungal mutualist partners (Hulcr *et al.* 2011) indicates strong coevolution and suggests that the use of volatile cues is important for the maintenance of close relationships between ambrosia beetles and their fungal symbionts.

1.5 FUNGI AS PATHOGENS & PREDATORS

Especially in eusocial insects, pathogen avoidance is of significant importance to prevent the spread of an infection through the whole nest via social interactions or infected cadavers of conspecifics that remain in the nest. Termites have different behavioural and physiological mechanisms to counter pathogen transmission (e.g. Rosengaus *et al.* 1998a; 1999; 1998b) and volatiles play a significant role in the detection of harmful microbes. Mburu *et al.* (2009) tested behavioural responses of termites to isolates of the pathogenic fungi *M. anisopliae* and *B. bassiana* of different virulence and found a positive correlation between isolate virulence and volatile-mediated repellency. Three compounds, namely 4,5-dihydro-5-pentyl-2(3H)-furanone, 2-pyrrolidinone, and borneol, were identified to contribute most to the repellency and higher concentrations of these compounds are suggested to be responsible for the higher repellency of more virulent strains (Mburu *et al.* 2013; 2011). In line with this, Yanagawa *et al.* (2011; 2015) have demonstrated that termites increased their grooming activity in the presence of conspecifics infected with different pathogenic fungi without direct contact and furthermore, found a deterrent effect of the oxylipin volatile 3-octanone, which is emitted by the pathogenic fungus *I. fumosorosea*. Therefore, the use of fungal volatile cues by termites can be assumed to be of crucial importance for an early detection of pathogens and represents an effective prevention strategy. In contrast to the well investigated volatile-mediated pathogen avoidance behaviour of termites, this aspect is poorly explored with respect to other invertebrate groups. Besides termites, only the parasitoid wasp *L. distinguendus* and the pine weevil *H. abietis* are known to use fungal volatiles to avoid contact with fungal pathogens (Azeem *et al.* 2013, Steiner *et al.* 2007). Other studies that focused on pathogen avoidance behaviour of herbivorous termites, beetles, aphids, and parasitoid wasps also found a deterrent effect of different pathogenic fungi (e.g. Cotes *et al.* 2015, Hussain *et al.* 2010, Ormond *et al.* 2011, Rashki and Hirvani 2013), however, from these studies it remains unclear whether behavioural decisions solely based on volatile information and whether fungal-induced plant volatiles were responsible for behavioural decisions rather than fungal volatiles per se.

Another type of antagonism involves predacious fungi and nematodes. Nematophagous fungi *A. oligospora* and *E. vermicola* employ different volatiles for attracting their prey (Hsueh *et al.* 2017, Lin *et al.* 2013). In contrast to most of the above described functions of fungal volatiles as infochemicals, here, the emission of certain volatiles can be considered as a specific signalling pathway evolved to fulfil the function as nematode attractants.

1.6 FUNGI IN A MULTITROPHIC CONTEXT

In multitrophic interactions fungal volatiles are used as cues by carnivorous and herbivorous predators as well as parasitoids during foraging to locate fungal-associated prey organisms. Preference tests by Hall and Hedlund (1999) and Pfeffer and Filser (2010) revealed that the predatory mite *H. aculeifer* was attracted by the volatile bouquet of two saprotrophic fungi but not by the volatile bouquet of its prey, fungivorous springtails. Both studies have also shown that previous grazing of fungal colonies by springtails did not increase attraction of *H. aculeifer*. The use of volatile cues that are typically emitted by prey-associated fungal food patches rather than the use of more specific cues originating from single prey species is suggested to be more efficient for generalist predators of fungivorous animals since it may increase the possibility to encounter more than one prey species (Pfeffer and Filser 2010). This also applies to parasitoid and predacious wasps, flies, and beetles (Table 1.1). A number of studies have demonstrated that parasitoid wasps take advantage of using volatile cues emitted by fungi that serve as hosts and/or niche constructors for the wasp's prey organisms, *Drosophila* fruit flies, beetles, and woodwasps (Boone *et al.* 2008, Dicke *et al.* 1984, Sullivan and Berisford 2004, Vet *et al.* 1984). Interestingly, females of the parasitoid wasp *I. leucospoides* are even able to use age-specific characteristics in the volatile profile of the fungal symbiont of their host *S. noctilio* for the location of suitably aged host larvae (Jofré *et al.* 2016).

1.7 CONCLUSION

Fungal volatiles play important roles as mediators of fungus-invertebrate interactions and fulfil multiple functions as attractants and deterrents; they are used as food-finding cue by foraging invertebrates, inform on the presence of suitable microhabitats (deadwood, fermented fruit substrates) for feeding and/or oviposition, mediate avoidance of fungal pathogens, are used as lure by predacious fungi to attract prey animals, and lead non-fungivorous parasitoids and predators to fungal-associated prey/host animals. The ubiquitous use of fungal volatiles as infochemicals by both above- and belowground invertebrates suggests a substantial involvement of volatiles in the maintenance of fungus-invertebrate interactions.

Two general patterns can be deduced from the here reviewed literature: first, insects that are

naturally associated with microbe/fungal pre-colonised plant substrates (e.g. deadwood, fermented fruits) rely on common ubiquitous compounds related to microbial activity and often deriving from yeast fungal colonisers - short-chain alcohols, acids, acetates, aldehydes - rather than on more specific compounds like terpenoids. Some studies suggest an involvement of insects in vectoring fungal propagules to new substrates/environments in respective systems, however, the current evidence is rather weak. Potential mutualistic characters of respective interactions need to be investigated in further detail, e.g. by measuring insect-related dispersal efficiencies and fitness consequences for both insects and fungi. The second pattern that appears from the literature relates to a certain group of fungal volatiles, namely oxylipins (eight-carbon compounds). Especially for deadwood-associated beetles, eight-carbon oxylipin volatiles (3-octanone, 1-octen-3-ol, 1-octanol, 3-octanol, etc.) are important infochemicals and significantly involved in the location and selection of (mutualist) host fungi. Furthermore, a few studies demonstrate that oxylipin volatiles mediate avoidance of fungal pathogens in hymenoptera and coleoptera by acting as deterrents. Plant-derived oxylipins are well known to play major roles as infochemicals in plant-herbivore systems, however, whether fungal-derived oxylipins are of equal importance in mediating fungus-invertebrate interactions, as it is suggested by Hanski (1989) and Holighaus and Rohlfs (2018) remains to be investigated in more detail by including other groups of fungus-related invertebrates.

Table 1.1: *Overview of volatile-mediated interactions between fungi and invertebrates with focus on animal behavioural responses to fungal volatiles, sorted by type of interaction and animal groups. Studies with focus on subjects of applied chemistry without reference to natural ecology are not included. (n.r.: no response, n.p.: no preference)*

Animal Species	Group	Odour Source (fungal species, VOC, material infected with fungi)	Mode of Action, Behavioural response	Experimental Setup	Reference
FUNGI AS DIET					
Tyrophagus putrescentiae	Acari	VOC identified from 15 Homobasidiomycetes: Cis- & trans-octa-1,5-dien-3-ol	Attraction	Olfactometer	Vanhaelen et al. (1980)
Archegozetes longisetosus *Scheloribates sp.*	Acari	Pairwise testing of: (a) Mixture of essential amino acids (b) Mixture of non-essential amino acids (c) Mixture of fatty acids (d) Mixture of D-glucose and 1-octen-3-ol	*A. longisetosus*: Preference for (c) *Scheloribates* sp.: Preference for (d)	Circular two-choice olfactometer (petri dish); contact to the volatile sources was probably possible groups of 10 individuals tested per run	Brückner et al. (2018)
Eisenia fetida	Annelida	(a) VOC filtrate of *Geotrichum candidum* VOCs identified from *G. candidum*: (b) Ethyl acetate (c) Ethyl propionate (d) Ethyl penanoate (e) Ethyl hexanoate (f) 3-octanone (g) 2-methyl-1-butanol (h) 3-methyl-1-butanol (i) 2-methyl-1-propanol	(a) Attraction (horizontal & vertical) (b) n.r. (c) n.r. (d) Attraction (horizontal) (1, 10, 100, 1000 µl) (e) Attraction (horizontal) (1, 100, 1000 µl; 10 µl: n.r.) (f) n.r. (g) n.r. (h) n.r. (i) n.r.	Horizontal four-arm olfactometer (below-ground, filled with compost soil); groups of 5-150 individuals tested per run Vertical olfactometer (below-ground, filled with compost soil); groups of 10 individuals tested per run	Zirbes et al. (2011)
Psyllobora vigintimaculata	Coleoptera	(a) Plants infected with *Podosphaera* sp. vs. uninfected plants VOCs identified from *Podosphaera* sp.: (b) 1-octen-3-ol (c) 3-octanone (d) 3-octanol (e) Linalool (f) Benzyl alcohol	(a) Infected plants were preferred over uninfected plants (b) 1 µg: attraction; 10 µg: attraction; 100 µg: attraction (c) n.r. (d) n.r. (e) n.r. (f) n.r.	Two-arm olfactometer with active airflow; choice experiment; 1 individual tested per run	Tabata et al. (2011)
Onychiurus armatus	Collembola	(a) *Verticillium bulbilosum* grown on malt extract agar (b) *Verticillium bulbilosum* grown on soil (c) *Penicillium spinolosum* grown on malt extract agar (d) *Penicillium spinolosum* grown on soil (e) *Mortierella isabellina* grown on malt extract agar (f) *Mortierella isabellina* grown on soil	(a) *V. bulbilosum* preferred over control, attraction (b) n.r. (c) n.r. (d) n.r. (e) n.r. (f) *M. isabellina* preferred over control, attraction	Two-arm olfactometer with active airflow; groups of 7 individuals tested per run; treatments tested against control (malt extract agar, soil)	Bengtsson et al. (1988)
Onychiurus armatus	Collembola	*M. isabellina* grown on soil	Attraction (from a distance of 40 cm)	One-arm olfactometer with 5 vials connected to each other & *M. isabellina* added to the farthest vial; groups of 60 individuals tested per run; direct contact to the odour source was possible	Bengtsson et al. (1994)

To be continued

Table 1.1: *Continuation*

Orchesella cincta *Tomocerus flavescens*	Collembola	(a) *Cladosporium herbarum* (b) *Mortierella isabellina* (c) *Cladosporium cladosporioides* (d) *Penicillium spinulosum*	(a) Attraction (*O. cincta)* (b) Attraction (*T. flavescens)* (c) n.r. (d) n.r.	Two-arm olfactometer with active airflow (1 individual)	Hedlund et al. (1995)
Onychiurus sinensis	Collembola	(a) *Alternaria alternata* (b) *Aureobasidium pullulans* (c) *Cladosporium cladosporioides* (d) *Epicoccum purpurascens* (e) *Trichothecium roseum* (f) *Mucor plumbeus* (g) *Penicillium spinulosum* (h) *Trichoderma polysporum*	Attractivity ranking treatment 1: (f) > (e) > (a) > (b) = (g) > (c) = (d) > (h) (repulsion) Attractivity ranking treatment 2: (e) = (c) = (f) = (d) > (b) > (a) = (g) = (h) Treatment 3: Preference for/attraction to (e), (d), (g), (f)	Circular two-choice olfactometer (petri dish) groups of 20 individuals tested per run; direct contact to the odour source was possible Treatment 1: pure fungal tissue Treatment 2: fungus + litter vs. control (litter) Treatment 3: fungal odour solution vs. control (filter paper)	Sadaka-Laulan et al. (1998)
Proisotoma minuta	Collembola	VOCs identified from Tricholoma matsutake: (a) 1-octen-3-ol (b) Methyl cinnamate	(a) Aggregation decreased with increasing concentration; aggregation totally inhibited at 1000 ppm (repellence) (b) 0.001, 0.01, 0.1 ppm: no inhibition of aggregation; 1, 10, 100 ppm: incomplete but significant inhibition 1000 ppm: strong inhibition of aggregation (repellence)	Circular olfactometer; groups of 70 individuals tested per run	Sawahata et al. (2008)
Folsomia candida	Collembola	Intimate mix of cow dung debris & feces	Attraction (from a distance of 25 mm)	Video observation; 1 individual tested per run; no-choice	Auclerc et al. (2010)
Folsomia candida *Heteromurus nitidus* *Supraphorura furcifera*	Collembola	(a) *Aspergillus nidulans* (WT) vs. *A. nidulans* (ΔlaeA) (b) *Cladosporium cladosporioides* vs. *A. nidulans* (WT) (c) *A. nidulans* (sterigmatocystin deficient) vs. *A. nidulans* (WT) (d) Ungrazed *A. nidulans* (WT) vs. ungrazed (e) Ungrazed *Laccaria bicolor* vs. ungrazed	Collembola generally attracted to fungi (a) *A. nidulans* (ΔlaeA) preferred (all species) (b) *C. cladosporioides* preferred (*H. nitidus* & *S. furcifera*) (c) *C. cladosporioides* preferred (*H. nitidus* & *S. furcifera*) (d) Ungrazed preferred *(F. candida* & *H. nititdus*) (e) Ungrazed preferred *(F. candida* & *H. nititdus*)	Four-chamber olfactometer; choice experiment; groups of 25 individuals tested per run; *C. cladosporioides was* tested as a 'high quality reference'	Staaden et al. (2011)
	Diptera Dermaptera	Blend of VOCs identified from *Lysurus mokusin*: Butanoic acid + p-cresol + phenol + indole	Flies: Attraction of Sacophagidae, Calliphoridae, Muscidae, Sepsidae, Drosophilidae Earwigs: n.r.	Field traps for catching flies and earwigs	Chen et al. (2014)
Ariolimax columbianus	Gastropoda	1-octen-3-ol (identified from *Clitophilus prunulus* with strongly increased amounts in wounded/crushed specimens) applied on leaves of lettuce (well accepted food)	Inhibited feeding (antifeedant) (0.01, 0.1, 1 ml; 0.83 g/ml)	Antifeedant experiment in glass plates	Wood et al. (2001)
Oniscus asellus	Isopoda	Moulded food (not further specified) vs. sterilized food	No volatile-mediated response, but preference for moulded food after contact	Video observation; four-chamber olfactometer; groups of 8 individuals tested per run; direct contact to the odour source was possible	Zidar et al. (2003)
Porcellio scaber	Isopoda	(a) Food with microorganisms (not further specified) (b) Food without microorganisms	(a) Attraction (b) n.r.	Eight-chamber olfactometer; 1 individual tested per run	Zimmer et al. (1996)
		VOCs identified from *Tuber* spp.: (a) dimethyl sulphide (b) 2-methyl propanal (c) 2-butanone (d) 2-methyl-butanol (e) 2-methyl-butanal (f) 3-methyl-butanol (g) 3-methyl-butanal	 (a) Attraction of mycetophilous & true hydnophagous insects (b) n.r. (c) n.r. (d) n.r. (e) n.r. (f) n.r. (g) Attraction of mycetophilous insects	Field traps for catching arthropods associated with *Tuber* spp.	Pacioni et al. (1991)

To be continued

Table 1.1: *Continuation*

FUNGI AS HOSTS					
Orizaephilus surinamensis *Orizaephilus mercator* *Cryptolestes ferrugineus* *Ahasverus advena* *Cathartus quadricollis*	Coleoptera	VOCs characteristic for fungi: (a) 3-octanol (racemic) (b) 3-octanone (racemic) (c) 3-methylbutanol (d) 2-phenylethanol (e) 1-octen-3-ol (racemic) (f) (*R*)-(-)-1-octen-3-ol (g) (*S*)-(+)-1-octen-3-ol	 (a) 0.01-10 µg: attraction (all species except *C. quadricollis*) (b) 0.01-100 µg: attraction (all species except *C. quadricollis*) (c) 0.01-1 µg: attraction (all species) (d) 2-phenylethanol (e) 0.01-10 µl: attraction (all species except *C. quadricollis*); (f) 0.0001 µg: n.r.; 0.001-10 µg: attraction; 100 µg: repulsion (g) 0.0001 µg: n.r.; 0.001-10 µg: attraction; 100 µg: repulsion	Circular two-choice pitfall olfactometer (petri dish); groups of 12 individuals tested per run; test against control (solvent)	Pierce et al. (1991)
	Coleoptera	*Fomitopsis pinicola* *Fomes fomentarius*	Attraction of 28 coleoptera taxa; attraction of coleoptera colonizing deadwood; strong attraction of Ciidae to their host *F. pinicola*	Field traps	Jonsell & Nordlander (1995)
	Coleoptera Lepidoptera	VOCs identified from wounded/chopped fruiting bodies of *Formitopsis pinicola* & *Fomes fomentarius*: (a) 1-octen-3-ol (racemic) (b) 3-octanone (racemic) (c) Blend: 3-octanone + 1-octen-3-ol (d) Blend: 1-octanol + 3-octanol + 1-nonanol + 1-octen-3-ol	 (a) Attraction of Coleoptera *Anaspis marginicollis, A. rufilabris,* & Lepidoptera *Ephinotia tedella* (b) n.r. (c) Attraction of *P. succicola, A. marginicollis* & *A. rufilabris* (d) Attraction of *A. rufilabris* & *E. tedella*	Field traps	Fäldt et al. (1999)
Octotemnus glabriculus *Cis boleti* *Cis nitidus* *Cis bilamellatus*	Coleoptera	(a) Fruiting bodies of *Coriolus versicolor* (b) VOC filtrate of *C. versicolor* (c) Fruiting bodies of *Ganoderma adspersum* (d) VOC filtrate of *G. adspersum* (e) Fruiting bodies of *Piptoporus betulinus* (f) VOC filtrate of *P. betulinus*	(a) Attraction of *C. bilamellatus*, *O. glabriculus* & *C. boleti* (*C. nititdus*: n.r.) (b) Attraction of *C. bilamellatus*, *O. glabriculus* & *C. boleti* (*C. nititdus*: n.r.) (c) Attraction of *C. bilamellatus* & *C. nititdus* (other species: n.r.) (d) Attraction of *C. bilamellatus* & *C. nititdus* (other species: n.r.) (e) Attraction of *C. bilamellatus* (other species: n.r.) (f) Attraction of *C. bilamellatus* (other species: n.r.)	Circular glass olfactometer with active airflow and the odour source placed outside at one side of the arena; 1 individual tested per run	Guevara et al. (2000a)
Octotemnus glabriculus *Cis boleti*	Coleoptera	(a) Young fruiting bodies of *C. versicolor* (b) Mature fruiting bodies of *C. versicolor*	(a) Attraction of *O. glabriculus* (*C. boleti*: n.r.) (b) Attraction of (both species)	Circular glass olfactometer with active airflow and the odour source placed outside at one side of the arena; 1 individual tested per run	Guevara et al. (2000b)
Bolitophagus reticulatus	Coleoptera	(a) *Fomes fomentarius* (b) Ethanol (c) *F. fomentarius* + ethanol	(a) Weak attraction (borderline significance) (b) Attraction (c) Attraction	Field traps	Jonsell et al. (2003)
Cis boleti	Coleoptera	(a) VOC filtrate of *Trametes gibbosa* (b) (*R*)-(-)-1-octen-3-ol (c) (*S*)-(+)-1-octen-3-ol	(a) Attraction (b) Attraction & increased searching activity (c) Attraction & increased searching activity	Circular two-choice olfactometer (petri dish); groups of 10 individuals tested per run; test against control (solvent)	Thakeow et al. (2008)
Tritoma bipustulata *Sulcaris affinis* *Diaperis boleti*	Coleoptera	(a) *Trametes versicolor* (b) VOC headspce sample of *T. versicolor* (c) 1-octen-3-ol (concentration: 10^{-2})	(a) Attraction of all species (b) Attraction of all species (c) Attraction of *T. bipustulata* & *S. affinis* (*D. boleti* not tested)	Two-choice olfactometer; groups of 10 individuals tested per run; treatments tested against control (medium, solvent)	Drilling & Dettner (2009)
Xyleborus glabratus *Xylosandrus crassiusculus*	Coleoptera	(a) *Raffaelea lauricola* (b) *Ambrosiella xylebori*	(a) Attraction of *X. glabratus* (symbiont); repulsion of *X. crassiusculus, X. saxesenii*	Two-choice olfactometer; groups of 20 individuals tested per run;	Hulcr et al. (2011)

To be continued

Table 1.1: *Continuation*

Xyleborus ferrugineus *Xyleborinus saxesenii*		(c) *Ambrosiozyma ambrosiae* (d) Ethanol	(b) Attraction of *X. ferrugineus, X. crassiusculus* (symbiont); repulsion of *X. glabratus* (c) Attraction of *X. glagratus, X. ferrugineus* (symbiont) (d) Attraction of *X. crassiusculus, X. ferrugineus*	test against control (medium, solvent)	
Bolitophagus reticulatus	Coleoptera	VOCs identified from *Fomes fomentarius*: (a) 3-octanone (10^{-3}) (b) 1-octen-3 ol (10^{-3}) (c) 3-octanol (10^{-3})	 (a) Attraction (b) Repellence (c) n.r.	Circular two-choice pitfall olfactometer (petri dish); groups of 6 individuals of the same sex tested per run; test against control (solvent)	Holighaus et al. (2014)
Botanophila spp.	Diptera	VOC identified from *Epichloë typhina* & *E. sylvatica*: Chokol K (racemic)	Attraction	Sticky field traps	Schiestl et al. (2006)
Botanophila spp.	Diptera	VOCs identified from *Epichloë clarkii, E. festucae & E. typhina:* Blend: Chokol K (racemic) + methyl (Z)-3- methyldodec-2-enoate	Attraction	Sticky field traps	Steinebrunner et al. (2008)
Lycoriella ingenua	Diptera	(a) *Agaricus bisporus* (b) *Trichoderma aggressivum*	(a) n.r. (b) Attraction	Circular two-choice pitfall olfactometer (glass petri dish)	Cloonan et al. (2016a)
Lycoriella ingenua	Diptera	(a) *Trichoderma aggressivum* vs. *Aspergillus niger* (b) *T. aggressivum* vs. *Aspergillus flavus* (c) *T. aggressivum* vs. *Aspergillus fumigatus* (d) *T. aggressivum* vs. *Penicillium citrinum* (e) *T. aggressivum* vs. *Scatylidium thermophilum* (f) *T. aggressivum* vs. *Chaetomium* sp.	(a) *T. aggressivum* preferred (b) *T. aggressivum* preferred (c) *T. aggressivum* preferred (d) n.r. (e) n.r. (f) *T. aggressivum* preferred	Circular two-choice pitfall olfactometer (glass petri dish); 1 individual tested per run	Cloonan et al. (2016b)
Aradus abtectus	Heteroptera	(a) Mycelium of *Fomitopsis pinicola* (b) Fruiting body of *Fomitopsis pinicola*	(a) Attraction (b) Repellence	Olfactometer (60 cm) with active airflow; no-choice assay; 1 individual tested per run	Koban et al. (2016)
Sirex noctilio	Hymenoptera	*Amylostereum areolatum* vs. *A. chailletii*	*A. areolatum* preferred by mated females	Walk-in flight tunnel two-choice olfactometer	Sarvary et al. (2016)
FUNGI AS 'NICHE CONSTRUCTORS' & COMPETITORS					
Carpophilus hemipterus *Carpophilus lugubris*	Coleoptera	Fruit substrate infected with *Saccharomyces cerevisiae*	Attraction increased compared with uninfected fruits	Flight tunnel olfactometer with active airflow; test against control (uninfected fruits)	Blackmer & Phelan (1991)
Carpophilus lugubris	Coleoptera	VOCs identified from fruits & bread dough inoculated with *Candida krusei, Saccharomyces cerevisiae*: (a) Synthetic bread dough blend: acetaldehyde + ethanol + 1-propanol + ethyl acetate + 2-methylpropanol + 3-methylbutanol + 2-methylbutanol (b) Blend 1: acetaldehyde + ethyl acetate (c) Blend 1 + ethanol (d) Blend 1 + 1-propanol (e) Blend 1 + 2-methylpropanol (f) Blend 1 + 3-methylbutanol (g) Blend 1 + 2-methylbutanol (h) Blend 2: acetaldehyde + 2-methylpropanol (i) Blend 2 + ethyl acetate	(a) Full blend: attraction; blend without any of the alcohols or ethyl acetate: attraction; blend without acetaldehyde: reduced attraction; blend without all alcohols: reduced attraction (b) n.r. Attractivity ranking: (a) = (e) > (f) = (g) > (d) > (c) Attractivity ranking: (a) = (i) ≥ (j) ≥ (k) ≥ (l) = (h)	Flight tunnel olfactometer with active airflow; groups of 10 individuals tested per run; test against control (empty)	Lin & Phelan (1991)

To be continued

Table 1.1: *Continuation*

		(j) Blend 2 + ethanol (k) Blend 2 + 1-propanol (l) Blend 2 + 3-methylbutanol + 2-methylbutanol (m) Blend 3: acetaldehyde + 2-methylpropanol + 3-methylbutanol (n) Blend 3 + ethyl acetate (o) Blend 3 + ethanol (p) Blend 3 + 1-propanol	Attractivity ranking: (a) = (n) = (o) > (m) = (p)		
Carpophilus hemipterus	Coleoptera	(a) *Saccharomyces cerevisiae* (b) Blend of VOCs identified from *S. cerevisiae* (1): acetaldehyde + ethanol + propanol + 2-butanone + ethyl acetate + 2-methylpropanol + butanol + 2-pentanone + 2-pentanol + 3-hydroxy-2-butanone + 3-methylbutanol + isobutyl acetate + ethyl butyrate + ethyl isovalerate + isopentyl acetate + isopentyl butyrate + isopentyl isovalerate (c) Blend of VOCs identified from *S. cerevisiae* (2): ethyl acetate + acetaldehyde + 2-pentanol + 3-methylbutanol	(a) Attraction (b) Attraction (c) Attraction	Flight tunnel olfactometer with active airflow; groups of 10 individuals tested per run; test against control (sterile fruit)	Phelan & Lin (1991)
Carpophilus hemipterus *Carpophilus lugubris* *Stelidota geminata*	Coleoptera	(a) *Ceratocystis fagacearum* (b) *Xerula radicata* (c) *Pluteus atricapillus* (d) *Tyromyces chioneus* (e) *Botrytis cinerea*	(a) Attraction (b) Attraction (c) n.r. (d) n.r. (e) n.r.	Flight tunnel olfactometer with active airflow; groups of 10 individuals tested per run; test against control (empty)	Lin & Phelan (1992)
Carpophilus humeralis	Coleoptera	(a) *Saccharomyces cerevisiae* (b) *Candida guilliermondii* (c) *Candida shehatae* (d) Blend of VOCs identified from *S. cerevisiae*: ethanol + acetaldehyde + 2-methylpropanol + 1-propanol + ethyl acetate + 2-methylbutanol (e) Blend of VOCs identified from *C. guilliermondii*: ethanol + 2-methylpropanol + 3-hydroxy-2-butanone (f) Blend: ethanol + 2-methylpropanol (g) 3-hydroxy-2-butanone	(a) Attraction (b) Attraction, but less compared to *S. cerevisiae, C. shehatae* (c) Attraction (d) Attraction (e) Attraction (f) n.r. (g) Attraction	Flight tunnel olfactometer with active airflow; groups of 500-1000 individuals tested per run	Nout & Bartelt (1998)
Xestobium rufovillosum	Coleoptera	(a) *Donkioporia expansa* (b) *Coriolus versicolor* (c) Wood infected with *D. expansa, C. versicolor, Fistulina hepatica*	(a) Attraction (b) Attraction (c) Attraction	Flight tunnel olfactometer with active airflow; groups of 5 individuals tested per run; test against control (empty)	Belmain et al. (2002)
Drosophila spp.	Diptera	Different yeast species	Different yeast spp. attracted different *Drosophila* spp.	Field traps	Dobzhansky et al (1956)
Drosophila melanogaster	Diptera	(a) Odour sample of fermented grape juice (*S. cerevisiae)* (b) Blend of *S. cerevisiae* characteristic VOCs: Acetoin + acetic acid + 3-methyl-1-butanol + 2-phenyl-ethanol +ethanol	(a) Attraction (b) Attraction	Flight tunnel olfactometer with active airflow; no-choice assay; groups of 20 female individuals tested per run;	Becher et al. (2012)

To be continued

Table 1.1: *Continuation*

Drosophila melanogaster	Diptera	(a) *Saccharomyces cerevisiae* (different isolates) (b) *Saccharomyces bayanus* (different isolates) (c) *Saccharomyces uvarum* (different isolates) (d) *Saccharomyces paradoxus* (different isolates) (e) *Saccharomyces kudriavezii* (f) *Hanseniaspora uvarum* (g) *Pichia kluyveri* (h) *Candida railenensis* (i) *Kluveromyces aestuarii* (j) *Kazachstania telluris* (k) *Kluveromyces polysporus* (l) *Torulaspora delbrueckii* (m) *Candida castellii* (n) *Kluveromyces thermotolerans* (o) *Zygosacharomyces mrakii* (p) *Saccharomyces* spp. vs non-*Saccharomyces* spp.	(a) Attraction: 13 isolates; n.r.: 1 isolate; repellence: 2 isolates (b) Attraction (c) Attraction: 3 isolates; n.r.: 1 isolate; repellence: 1 isolate (d) Attraction: 2 isolates; n.r.: 2 isolates; repellence: 1 isolate (e) Attraction (f) Attraction (g) Attraction (h) n.r. (i) n.r. (j) n.r. (k) n.r. (l) n.r. (m) Repellence (n) Repellence (o) Repellence (p) *Saccharomyces* spp. preferred	Two-choice olfactometer; groups of 70-80 individuals tested per run; test against control (medium: sterile grape juice)	Palanca et al. (2013)
Drosophila simulans	Diptera	Odour sample of fermented grape juice (different genotypes of *Saccharomyces cerevisiae*)	Attraction (27 genotypes); repulsion (5 genotypes)	Two-arm olfactometer; groups of 3 individuals tested per run; test against control (medium: sterile grape juice)	Buser et al. (2014)
Drosophila melanogaster	Diptera	(a) *Candida californica* (b) *Penicillium expansum* (vegetative) (c) *Penicillium expansum* (sporulating) VOCs identified from *C. californica:* (d) Propanoic acid (e) 2-methylbutanoic acid (f) 2-methylpropanoic acid (g) 3-methylbutanoic acid VOCs identified from *C. californica* & *P. expansum*: (h) 3-methyl-1-butanol (i) 2-methyl-1-propanol VOCs identified from *P. expansum*: (j) 2-methyl-1-butanol (k) 3-methyl-3-buten-1-ol (l) 2-methyl-3-buten-2-ol (m) Furan (n) 3-methyl furan (o) (±)-beta-pinene (p) 2-methylisoborneol (q) (±)-Geosmin (r) Blend of VOCs identified from *C. californica*: 3-methyl-1-butanol + propanoic acid + 2-methyl-butanoic acid (s) Blend of VOCs identified from *P. expansum*: 3-methyl-1-butanol + 2-methyl-1-butanol + 3-methyl-3-buten-1-ol	(a) Attraction (b) Attraction (c) Attraction (d) 10^{-1}: attraction; 10^{-4}: n.r. (e) 10^{-1}: attraction; 10^{-4}: n.r. (f) n.r. (g) n.r. (h) 10^{-1}: attraction; 10^{-4}: n.r. (i) n.r. (j) 10^{-1}: attraction; 10^{-4}: n.r. (k) 10^{-1}: attraction; 10^{-4}: n.r. (l) n.r. (m) n.r. (n) n.r. (o) n.r. (p) n.r. (q) n.r. (r) 10^{-1}, 10^{-2}, 10^{-3}: Attraction (s) 10^{-1}, 10^{-2}: Attraction; 10^{-3}: n.r.	Circular olfactometer; 1 individual tested per run; no-choice assay	Stötefeld et al. (2015)

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Table 1.1: *Continuation*

Drosophila melanogaster *Drosophila suzukii*	Diptera	*Saccharomyces cerevisiae* *Hanseniaspora uvarum* *Pichia terricola* *Pichia kluyveri* *Candida californica* *Candida zemplinina*	Attraction of both *Drosophila* spp. to all yeast species; Strongest attraction of *D. melanogaster* to *H. uvarum* & *P. terricola*; Strongest attraction of *D. suzukii* to *H. uvarum*	Two-choice olfactometer; groups of 20 & 60 individuals tested per run; test against control (medium)	Scheidler et al. (2015)
Drosophila melanogaster	Diptera	(a) *Saccharomyces cerevisiae* (b) *Hanseniaspora uvarum* (c) *Candida californica* (d) *Pichia membranifaciens*	(a) Attraction (b) Attraction (c) Attraction (d) Attraction	Two-arm olfactometer; groups of 40-130 individuals tested per run; test against control	Fischer et al (2017)
Lobesia botrana	Lepidoptera	Grapes infected with *Botrytis cinerea* vs. uninfected grapes	Infected grapes preferred	Eight-chamber olfactometer with active airflow; groups of 35 larvae tested per run	Mondy et al. (1998)
Lobesia botrana	Lepidoptera	(a) Grapes infected with yeasts: *Saccharomyces cerevisiae, Zygosaccharomyces rouxii, Metschnikowia pulcherrima, Hanseniaspora uvarum, Pichia anomala* (b) Grapes infected with *Botrytis cinerea*	(a) Attraction, preferred oviposition site (b) Repellence, reduced oviposition	Two-choice olfactometer oviposition assay; 1 individual tested per run; test against control (uninfected grapes)	Tasin et al. (2011)
Cydia pomonella	Lepidoptera	*Metschnikowia andauensis*	Attraction	Flight tunnel olfactometer with active airflow; groups of 10 females tested per run; test against control	Witzgall et al. (2012)
FUNGI AS PATHOGENS & PREDATORS					
Hylobius abietis	Coleoptera	VOCs identified from *P. expansum:* (a) Styrene + pine twigs (b) 3-methylanisole + pine twigs	(a) Reduced attraction compared to pine twigs without styrene (b) Reduced attraction of males compared to pine twigs without 3-methylanisole	Circular multi-choice pitfall olfactometer; groups of 50 male & female individuals tested per run; test against controls (empty, fresh pine twigs)	Azeem et al. (2013)
Drosophila melanogaster	Diptera	Geosmin + Vinegar	Geosmin reduced attraction to vinegar	Flight tunnel olfactometer with active airflow; groups of 20 female individuals tested per run; test against control (distilled water)	Becher et al. (2010)
Musca domestica	Diptera	VOCs identified from potential pathogenic fungi *Phoma* sp., *Rhizopus* sp., *Fusarium* sp.: (a) Dimethyl trisulfide (b) 2-phenylethanol (c) Citronellal (d) Norphytone	 (a) Reduction of oviposition activity (b) Reduction of oviposition activity (c) n.r. (d) n.r.	Two-choice olfactometer oviposition assay; groups of 40 individuals tested per run; test against control (empty)	Lam et al (2010)
Lariophagus distinguendus (parasitoid of *Sitophilus granarius*)	Hymenoptera	(a) *Aspergillus sydowii* vs. control (medium: grain) (b) *Aspergillus versicolor* vs. control (medium: grain) (c) Larval feces with fungi vs. control (filter paper) (d) Fungal-free larval feces vs. control (filter paper) (e) Larval feces + fungi vs. fungal-free feces (f) 1-octen-3-ol vs. control (solvent)	(a) Avoidance of *A. sydowii* (b) Avoidance of *A. versicolor* (c) Avoidance of larval feces with fungi (d) Fungal-free larval feces preferred (e) Fungal-free larval feces preferred (f) Avoidance of 1-octen-3-ol	Four-chamber olfactometer; 1 individual tested per run	Steiner et al. (2007)
Macrotermes michaelseni	Isoptera	*Metarhizium anisopliae* (different isolates) *Beauveria bassiana* (different isolates)	Repellence; positive correlation of isolate virulence & repellence; *M. anisopliae* more virulent & repellent than *B. bassiana*	Two-arm olfactometer with active airflow; test against control (empty)	Mburu et al. (2009)

To be continued

Table 1.1: *Continuation*

Macrotermes michaelseni	Isoptera	(a) Blend of VOCs identified from *Metarhizium anisopliae* (highly repellent & virulent isolate): Hexanol + 3-octanone + acetic acid + 1-octene + 2-nonanone + 2-nonanol + phenylethyl alcohol + 3-nonen-2-one + borneol + 4,5-dihydro-5-pentyl-2(3H)furanone (in addition, 9-component blends with each compound substracted one at a time were tested) (b) Blend of VOCs identified from *Metarhizium anisopliae* (less repellent & virulent isolate): Hexanol + 3-octanol + n-ethylacetamide + 1-octen-3-ol + cedrene + butylolactone + phenylethyl alcohol + 1-ethyl-2-methylbenzene + 2-propyl-1-pentanol + 2-pyrrolidinone	(a) More repellent than (b); 4,5-dihydro-5-pentyl-2(3H)furanone contributed most to the repellency of the highly virulent isolate; 2-pyrrolidinone contributed most to the repellency of the less virulent isolate	Two-arm olfactometer with active airflow	Mburu et al. (2011)
Coptotermes formosanus	Isoptera	VOC solutions obtained from conidia of *Metarhizium anisopliae* (highly virulent and less virulent isolate) *Beauveria brongniartii* (highly virulent and less virulent isolate) *Isaria fumosorosea* (highly virulent and less virulent isolate) applied on conspecifics	Increased grooming activity in the presence of fungi	Observation of grooming behaviour with photographs; groups of 5 individuals tested per run; test against control	Yanagawa et al. (2011)
Coptotermes formosanus	Isoptera	*Metarhizium anisopliae* (highly virulent and less virulent isolate) *Beauveria brongniartii* (highly virulent and less virulent isolate) *Isaria fumosorosea* (highly virulent and less virulent isolate)	Avoidance of all species & isolates except from the less virulent isolate of *M. anisopliae*	Two-choice olfactometer; test against control; 1 individual tested per run	Yanagawa et al. (2012)
Macrotermes michaelseni	Isoptera	(a) Blend of VOCs identified from *Beauveria bassiana* (highly repellent & virulent isolate): Hexanol + (*R*,*S*)-4,5-Dihydro-5-pentyl-2-(3 H)furanone + 3-octanol + 1-pentanol + camphor + butyrolactone + (*R*,S)-borneol + 4-nonanone + octanol + 2-nonanone (in addition, 9-component blends with each compound substracted one at a time were tested) (b) Blend of VOCs identified from *Beauveria bassiana* (less repellent & virulent isolate): Hexanol + (*R*,*S*)-4,5-Dihydro-5-pentyl-2-(3 H)furanone + 3-octanol + 1-pentanol + camphor + butyrolactone + (*R*,S)-borneol + 4-nonanone + octanol + 2-nonanone (different amounts of compounds between blends (a) & (b))	(a) More repellent than (b); 4,5-dihydro-5-pentyl-2(3H)furanone contributed most to the repellency of both isolates, with higher amounts in the more repellent & virulent isolate; Borneol also strongly contributed to repellency of *B. bassiana*, and is also more abundant in the more virulent isolate	Two-arm olfactometer with active airflow	Mburu et al. (2013)
Coptotermes formosanus	Isoptera	(a) Conidia suspension of *Isaria fumosorosea* VOCs identified from *I. fumosorosea*: (b) 3-octanone (c) 1-octen-3-ol	(a) Avoidance (positively correlated with concentration) (b) Avoidance (c) n.r.	Two-choice olfactometer; test against control (solvent); 1 individual tested per run	Yanagawa et al. (2015)
Lobesia botrana	Lepidoptera	(a) Grapes infected with *Botrytis cinerea* VOCs identified from *Botrytis cinerea* infected grapes: (b) Ethanol (c) 3-methyl-1-butanol (d) 3-methyl-1-butanol in the presence of grapes	(a) Repellence, reduced oviposition (b) n.r. (c) 1 µg: n.r.; 10 µg: enhanced oviposition; 100 µg: n.r. (d) 1 µg: n.r.; 10 µg: enhanced oviposition; 100 µg: n.r.	Flight tunnel olfactometer with active airflow; groups of 10 mated females tested per run Two-choice olfactometer oviposition assay; 1 individual tested per run test against control (uninfected grapes, solvent)	Tasin et al. (2012)

To be continued

Table 1.1: *Continuation*

Epiphyas postvittana	Lepidoptera	(a) Grapes infected with *Botrytis cinerea*	(a) Repellence	Flight tunnel olfactometer with active airflow;	Rizvi & Raman (2016)
		VOCs identified from *Botrytis cinerea* infected grapes:		groups of 4 males or females tested per run	
		(b) Ethanol	(b) 10, 100, 1000 µg: n.r.	Two-choice olfactometer oviposition assay	
		(c) 3-methyl-1-butanol	(c) 10 µg: attraction, enhanced oviposition; 100 µg: n.r.	test against control (solvent)	
		(d) Ethanol in the presence of grapes	(d) 10, 100 µg: n.r.; 1000 µg: repellence, reduced oviposition		
		(e) 3-methyl-1-butanol in the presence of grapes	(e) 10 µg: n.r.; 100 µg: repellence, reduced oviposition		
Caenorhabditis elegans	Nematoda	(a) *Arthrobotrys oligospora* (nematophagous)	(a) Attraction	Circular two-choice olfactometer; test against control;	Hsueh et al. (2017)
		VOCs identified from *Arthrobotrys oligospora*:		groups of individuals tested per run;	
		(b) Dimethyl disulfide	(b) Moderate attraction	test against control (solvent, medium)	
		(c) (±)-2-methyl-1-butanol	(c) Strong attraction		
		(d) 2,4-dithiapentane	(d) Strong attraction		
		(e) Methyl 3-methyl-2-butenoate	(e) Strong attraction		
		(f) S-methyl thioacetate	(f) Strong attraction		
Bursaphelenchus xylophilus	Nematoda	(a) VOC filtrate of *Esteya vermicola* (nematophagous):	(a) Attraction	Two-choice olfactometer; test against control (solvent);	Lin et al. (2013)
		VOCs identified from *E. vermicola:*		groups of ~200 individuals tested per run	
		(b) Alpha-pinene	(b) 50 ng: attraction; 28 ng: n.r.		
		(c) Beta-pinene	(c) 12 ng: attraction; 6 ng: n.r.		
		(d) Camphor	(d) 1.6 ng: attraction; 1 ng: n.r.		
		(e) Blend: (b) + (c) + (d)	(e) 41 ng: attraction; 20.5 ng: n.r.		
FUNGI IN A MULTITROPHIC CONTEXT					
Hypoaspis aculeifer	Acari	(a) *Alternaria alternata* vs. control (medium)	(a) *A. alternata* preferred	Circular two-choice olfactometer (petri dish)	Hall & Hedlund (1999)
(predacious; prey: collembola)		(b) *A. alternata* vs. *A. alternata* grazed by collembola (prey)	(b) n.p.		
		(c) *A. alternata* vs. medium + collembola odour	(c) *A. alternata* preferred		
Hypoaspis aculeifer	Acari	(a) *T. viride* vs. control (medium)	(a) *T. viride* preferred	Two-choice olfactometer filled with soil;	Pfeffer & Filser (2010)
(predacious; prey: collembola)		(b) *T. viride* vs. *T. viride* grazed by collembola (prey)	(b) n.p.	groups of 30 individuals tested per run	
		(c) *T. viride* vs. collembola	(c) n.p.		
		(d) *T. viride* grazed by collembola vs. control	(d) *T. viride* grazed by collembola preferred		
		(e) *T. viride* grazed by collembola vs. collembola	(e) n.p.		
		(f) Collembola vs. control	(f) n.p.		
Araecerus fasciculatus	Coleoptera	(a) *Kluyveromyces lactis*	(a) Attraction	Two-arm olfactometer with active airflow;	Yang et al (2017)
(herbivorous)		VOCs identified from *K. lactis*		1 individual tested per run;	
		(b) 3-methylbutyl acetate	(b) 0.1-1000 µg/ml: n.r.	test against control (medium, solvent)	
		(c) 2-methylbutyl actetate	(c) 0.1-1000 µg/ml: n.r.		
		(d) furfuryl acetate	(d) 100, 1000 µg/ml: repellence; 0.1-10 µg/ml: n.r.		
		(e) 2-phenylethanol	(e) 10-1000 µg/ml: attraction; 0.1, 1 µg/ml: n.r.		
		(f) 2-phenylethyl acetate	(f) 10-1000 µg/ml: attraction; 0.1, 1 µg/ml: n.r.		
		(g) 2-phenylethyl propionate	(g) 10-1000 µg/ml: attraction; 0.1, 1 µg/ml: n.r.		
		(h) 2-phenylethyl isobutyrate	(h) 10-1000 µg/ml: attraction; 0.1, 1 µg/ml: n.r.		
		(i) 2-phenylethyl butyrate	(i) 100, 1000 µg/ml: attraction; 0.1-10 µg/ml: n.r.		

To be continued

Table 1.1: *Continuation*

Ibalia leucospoides (parasitoid of *Sirex noctilio*)	Hymenoptera	*Amylostereum areolatum*	Antenna movement was identical to that observed in repsonse to suitably aged prey & parasitoids adopted searching in response to the fungal odour source (antennae lowered)	Direct observation of antenna movement of female individuals	Madden (1968)
Asobara tabida (isolated from *S. cerevisiae* & *Beta vulgaris* microhabitats) (parasitoid of *D. melanogaster*)	Hymenoptera	*Saccharomyces cerevisiae*	Attraction of *A. tabida* from the yeast microhabitat (*A. tabida* from the beet habitat: n.r.)	Four-arm olfactometer with active airflow (first choice);	Vet et al. (1984)
Leptophilina heterotoma (parasitoid of *D. melanogaster*)	Hymenoptera	(a) *Saccharomyces cerevisiae* + host (b) *S. cerevisiae* (c) Host (d) Dead *S. cerevisiae* vs. living *S. cerevisiae* (e) *S. cerevisiae* + sugar vs. *S. cerevisiae* VOCs identified from *Saccharomyces cerevisiae*: (f) Ethanol (g) Acetaldehyde (h) Ethyl acetate (i) Acetic acid (j) Formaldehyde (k) Methanol (l) Diacetyl (m) Ethanol + yeast (n) Acetaldehyde + yeast (o) Blend: ethanol + acetaldehyde + ethyl acetate vs. yeast	(a) Attraction (b) Attraction (c) n.r. (d) Living yeast preferred, attraction (e) n.p. (f) Attraction (g) Attraction (h) Attraction (i) n.r. (j) n.r. (k) n.r. (l) n.r. (m) Attraction stronger compared to ethanol alone (n) Attraction stronger compared to yeast & acetaldehyde alone (o) Blend less attractive than yeast *S. cerevisiae*	Video observation in an olfactometer with active airflow; first, second, final choice observed to detect arresting; 1 female individual tested per run	Dicke et al. (1984)
Roptrocerus xylophagorum *Spathius pallidus* (parasitoids of Scolytidae)	Hymenoptera	(a) *Ophiostoma ips* (b) *Ophiostoma minus*	(a) Attraction (b) Attraction	Two-arm olfactometer with active airflow (*R. xylophagorum*); Flight tunnel olfactometer with active airflow (*S. pallidus*); 1 female individual tested per run test against control (clean air, uninfected pine bold)	Sullivan & Berisford (2004)
Predators & parasitoids of *Ips pini*	Hymenoptera Diptera	(a) *Ophiostoma ips* (b) *Pichia scolyti*	(a) Attraction of parasitoid Hymenoptera, predacious Diptera (b) Attraction of predacious Diptera	Sticky field traps	Boone et al. (2008)
Vespula pensylvanica *Vespula germanica* (predacious)	Hymenoptera	(a) *Aureobasidium pullulans* VOCs identified from *A. pullulans*: (b) 2-methyl-1-butanol (c) 3-methyl-1-butanol (d) 2-phenylethanol (e) Blend: (b) + (c) + (d) (51:39:10% ratio)	(a) Attraction of wasps, mainly *Vespula pensylvanica* & *V. germanica* (b) Attraction (c) Attraction, less compared to (b), (e) (d) n.r. (e) Attraction	Field traps; comparison against control (medium, blank)	Davis et al. (2012)
Prorops nasuta (parasitoid of *Hypothenemus hampei)*	Hymenoptera	VOCs identified from fungal infected dust/frass of *H. hampei*: (a) 1-octen-3-ol (b) 3-octanone	 (a) n.r. (b) Attraction	Two-arm olfactometer; test against control (clean air); 1 female individual tested per run	Román-Ruíz et al. (2012)
Ibalia leucospoides (parasitoid of *Sirex noctilio*)	Hymenoptera	*Amylostereum areolatum*	Attraction to 14 day-old *A. areolatum*; n.r. to 5, 10, 17, 21, 24, 30 day-old *A. areolatum*	Two-arm olfactometer; 1 female individual tested per run; test against control (medium: malt-yeast-pine extract agar)	Jofré et al. (2016)

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SUBJECT OF THE PRESENT PHD PROJECT

A major challenge in terrestrial ecology is to understand the mechanisms underlying patterns of variation in foraging and feeding decisions of fungivorous soil arthropods, and hence how particular fungi are affected by grazers and vice versa. Increasing experimental evidence suggests a significant role of fungal volatiles, especially oxylipins, in shaping the foraging behaviour of fungivorous arthropods (Chapter 1), however, a direct link between the obvious dynamics in fungal volatile formation and the behaviours underlying arthropod foraging and feeding decisions has not yet been established. To gain more general insights into the chemical ecology of fungus-arthropod interactions, I analyse fungal volatile profiles by means of gas chromatography-mass spectrometry and observe behavioural responses of different Collembola species and isopods by focussing on both the searching and contact phase of the food selection process to characterise the mode of action of fungal volatiles, oxylipins in particular, as attractants, repellents, arrestants, deterrents and/or phagostimulants. By combining fungal chemistry and arthropod behaviour, I expect to provide important hints for a better understanding of the mechanisms that regulate the location of fungal food sources in Collembola and isopods, both major groups of facultative fungivores in decomposer systems.

Hypothesis 1: Based on the presumption that oxylipin volatiles are ubiquitous in soil systems, derived from the fact that the wound-activated emission of oxylipins is a conserved mechanism in higher fungi, I hypothesise that oxylipin volatiles function as food-finding cues for soil animals by acting as attractants, arrestants, and/or phagostimulants.

Hypothesis 2: Unicellular yeasts and filamentous fungi differ in their volatile emissions and Collembola are able to use characteristics in volatile profiles to differentiate between suitable and less suitable fungal species. In particular I expect that volatile-mediated attraction to or avoidance of fungal colonies during the searching phase are reflected in the acceptance or rejection of fungi during the contact phase and finally in an increased or reduced growth and reproduction of Collembola when fungi are given as single diets.

CHAPTER 2

WOUND-ACTIVATED FUNGAL OXYLIPIN VOLATILES FUNCTION AS INFOCHEMICALS FOR COLLEMBOLA

2.1 ABSTRACT

Fungivorous arthropods have been suggested to use fungal volatiles and in particular oxylipins as infochemicals to make adaptive foraging and feeding decisions in a similar way to herbivores. Fungal tissue wounding is well known to activate the emission of volatile oxylipin compounds, however, the effect of wounding-related chemical variation on the foraging behaviour of fungivorous soil arthropods is largely unknown and therefore represents the main issue of this study. Using a direct observation approach, I linked behavioural responses of individual Collembola (*Folsomia candida*, *Sinella curviseta*, *Heteromurus nitidus*) during the searching (food location) and contact phase (food acceptance) of food selection to wound-activated changes in the volatile profile of the dietary saprotrophic fungus *Chaetomium globosum*.

GC-MS analysis revealed a significant increase in headspace concentrations of the oxylipin volatiles 3-octanone and 3-octyl acetate in artificially wounded *C. globosum* colonies. Collembola movement patterns (volatile-mediated spatial foraging) were not affected by wounding, indicating that increased oxylipin emissions did not increase the attractivity of *C. globosum* colonies. In contrast, observation of feeding and contact behaviour revealed increased acceptance of wounded colonies as food source. Among the three tested species, the clearest response was observed in *H. nitidus* and *F. candida*; a significantly higher number of individuals fed on wounded colonies more rapidly and the mean duration of colony contacts was increased. Moreover, the proportion of time *H. nitidus* individuals spent with colony-contact and feeding was higher in the presence of wounded colonies. Higher acceptance of wounded colonies by *S. curviseta* is indicated by a lower number of colony contacts prior to the onset of feeding. In addition, the ability of the oxylipin compound 3-octanone to induce

'substrate biting', a here newly described component of the Collembola foraging behaviour, was tested. The number of *F. candida* and *S. curviseta* individuals that showed 'substrate biting' behaviour increased with increasing 3-octanone concentration (w/w 0-10^{-3}). I suggest that 'substrate biting' is a kind of exploratory behaviour that enables Collembola to test if fungal food is present, and can therefore be considered as 'test-biting' (probing).

Overall, the results strongly suggest that the wound-activated fungal oxylipin 3-octanone functions as food-finding cue and phagostimulant. Observed differences in behavioural responses of the three tested Collembola species are discussed in relation to species-specific resource use and species coexistence.

Keywords: *Collembola, fungus-arthropod interaction, spatial foraging behaviour, attraction, arrestance, test-biting, phagostimulant, infochemicals, volatile organic compounds, wounding, oxylipins, 3-octanone, 3-methyl-1-butanol*

2.2 INTRODUCTION

The perception and use of volatile organic compounds as infochemicals is most relevant in enabling arthropods to locate suitable feeding and/or oviposition sites from a distance. Numerous studies on plant-insect interactions have shown that volatiles, most notably oxylipins or 'green leaf volatiles', play a significant role in regulating host selection by herbivores (Ameye *et al.* 2017, Bruce *et al.* 2005). For example, plant volatile oxylipins have central function in a plant's defence response to herbivore grazing (Heil 2014) and are used as infochemicals by foraging insects to find their host plants (Bruce *et al.* 2005).

A long-standing supposition is that fungivorous arthropods use fungal borne chemicals to make adaptive foraging and feeding decisions in similar ways to herbivores (e.g. Rohlfs and Churchill 2011, Speed *et al.* 2012), and fungal-derived volatiles are strongly suspected to play a role in mediating fungus-arthropod interactions. In particular, fungal volatile oxylipins, which can be considered as analogous to plant oxylipins due to a common synthesis pathway (Hernández-Oñate and Herrera-Estrella 2015, Tsitsigiannis and Keller 2007), are suggested to function as host cues, attractants, repellents, arrestants, stimulants or deterrents for fungivorous arthropods (Hanski 1989). Preferences of fungivorous arthropods for certain fungi base on characteristics in fungal volatile profiles (Bengtsson *et al.* 1991),

and especially fungal volatile oxylipins have been demonstrated to influence fungivore foraging decisions by eliciting avoidance, attraction and/or arrestance (Bengtsson *et al.* 1991, Drilling and Dettner 2009, Fäldt *et al.* 1999, Holighaus *et al.* 2014, Pierce *et al.* 1991b, Sawahata *et al.* 2008, Tabata *et al.* 2011, Thakeow *et al.* 2008, Vanhaelen *et al.* 1980). Similar to plant oxylipins, fungal oxylipins are released in response to environmental stress, such as tissue wounding (Fäldt *et al.* 1999, Wurzenberger and Grosch 1983). As a consequence of their immobility, fungi are prey to a wide range of fungivorous soil animals and constantly exposed to mechanical feeding damage. There is direct and indirect evidence that artificial or grazer-mediated wounding of fungal tissue induces changes in the foraging behaviour of fungivorous soil arthropods (Döll *et al.* 2013). As every grazing event may have the potential to change the fungal volatile profile, facilitate food evaluation by the respective animal, and food-finding by other fungivorous soil animals moving in slightly further distance, the present study aims at directly linking foraging and feeding decisions to wounding-related variation in fungal volatile emissions using a direct observation approach.

According to Schoonhoven *et al.* (2005) the food selection process comprises two main phases, the searching phase followed by the contact phase, resulting in either acceptance or rejection of the potential food source. Whereas the searching behaviour is assumed to be exclusively driven by volatiles, additional visual and gustatory information may become relevant during the contact phase and could be used by foraging animals for the evaluation of a potential food source (Schoonhoven *et al.* 2005). To investigate foraging decisions with respect to these two phases of food selection, I chose a model system comprising *Folsomia candida*, *Sinella curviseta*, and *Heteromurus nitidus* Collembola, and the saprotrophic filamentous fungus *Chaetomium globosum*. Collembola are a well-known group of facultative fungivorous soil arthropods (Hopkin 1997) and have traditionally been used to investigate grazing preferences in soil animals (e.g. Bardgett *et al.* 1993, Heděnec *et al.* 2013, Hiol Hiol *et al.* 1994, Men'ko *et al.* 2006, Sadaka-Laulan *et al.* 1998, Visser and Whittaker 1977). Numerous short- and long-term choice experiments have shown that Collembola can be highly selective when foraging on saprotrophic fungi and are able to differentiate between fungal species (Bengtsson *et al.* 1991, Jørgensen *et al.* 2005, Maraun *et al.* 2003, Shaw 1988, Staaden *et al.* 2011). Preferences usually correlate positively with animal fitness measures (Chen *et al.* 1995, Heděnec *et al.* 2013, Rohlfs *et al.* 2007, Scheu and Simmerling 2004). Recent studies strongly suggest a significant role of variation in fungal secondary metabolite production in affecting the foraging behaviour and food choice of Collembola (Stötefeld *et al.* 2012, Yin *et al.* 2012). The use of fungal-derived volatiles by Collembola during spatial foraging has

been demonstrated by Hedlund *et al.* (1995). To extend our knowledge on the mechanisms driving fungus-arthropod interactions, I investigate the effect of fungal tissue wounding on the food-finding ability (off-patch searching) and feeding decision (on-patch behaviour) of Collembola. As *C. globosum* is known to serve as dietary fungus for different invertebrate soil animals such as lumbricids (Maraun *et al.* 2003), isopods (Ihnen and Zimmer 2008, Rothe and Gleixner 2000) and Collembola (Haubert *et al.* 2011), I hypothesise that *C. globosum*-derived volatile oxylipins function as cues for Collembola and consequently, that a wound-activated increase in volatile oxylipins increase the attractiveness of *C. globosum* colonies for foraging Collembola. Hence, Collembola are expected to be more strongly attracted to and/or arrested by volatiles of wounded compared to unwounded *C. globosum* colonies and higher attractivity results in higher acceptance of respective colonies as food source.

2.3 MATERIAL AND METHODS

2.3.1 CULTIVATION OF COLLEMBOLA AND *C. globosum*

Collembola, *F. candida* ('Berlin' strain), *S. curviseta*, and *H. nitidus* were cultured on a moistened mixture of plaster and activated charcoal (proportion 40:1) in Petri dishes (9 cm in diameter) with a vent in the lid that was covered with fine-mesh gauze. The lid was sealed with parafilm to prevent animals from escaping. Cultures were incubated at 20°C in constant darkness and fed with dried baker's yeast and/or freeze-dried stinging nettle powder once per week. Age synchronized populations were obtained by transferring adult individuals to new dishes for oviposition. After seven days adults were removed and eggs remained. For the experiments the Collembola were raised until the adult instar and their age at the time of experimental observation ranged between 60 and 90 days. Prior to the experiments the Collembola were starved for seven days. Fresh fungal colonies of *C. globosum* were obtained by cutting and transferring square pieces of fungal tissue (3x3 mm) to sterilized malt-extract agar (MEA; recipe: 6 g of agar, 9 g of malt extract, 1.5 g of soy peptone, filled up to 300 ml with H_2O) and incubation at 20°C in constant darkness.

2.3.2 SAMPLING, IDENTIFICATION, AND QUANTIFICATION OF *C. globosum* VOLATILES

Volatile profiles of *C. globosum* were determined to find out whether fungal tissue wounding affects volatile emissions. *C. globosum* fungal colonies were grown for five days in glass tubes (150 mm in height, 30 mm in diameter) filled with 50 ml MEA. The headspace solid-phase microextraction technique (HS-SPME; fibre: 85 μm CarboxenTM/ Polydimethylsiloxane StableFlexTM) (Pawliszyn 1997) was used to extract *C. globosum* volatiles from the fungal headspace at 20°C in darkness. Volatiles were collected from unwounded and wounded fungal colonies (8 replicates per treatment), and fungal-free MEA controls (4 replicates). Wounding of fungal colonies was done by standardized disruption of fungal tissue with a thin wire loop (3 mm in diameter) by scratching a mesh pattern (four horizontal and vertical scratches; approximately 50% of the fungal tissue was disrupted) exactly 30 minutes before the volatile extraction. After the wounding treatment glass tubes were sealed with parafilm, so that volatiles could accumulate for 30 minutes. Samples of unwounded *C. globosum* and MEA controls were treated in the same way except for the disruption of fungal tissue. The needle of the SPME device was carefully stuck through the parafilm into the glass tube and the fibre was exposed to the headspace over a period of 60 minutes. Volatile samples were successively analysed by using a gas chromatograph (GC; Agilent Technologies, 6890N; Palo Alto, USA) (non-polar HP-5ms column; 0.25 mm ID; 30 m length; 0.25 μm film thickness) coupled to a mass spectrometer (MS; Agilent Technologies, 5973N; Palo Alto, USA). The GC was programmed as follows: flow rate of 1.0 ml per minute (Helium as carrier gas), initial oven temperature -30 °C (cooled with liquid N_2) for 1.5 minutes, temperature ramp (6 °C minute-1 up to 200 °C), maintenance at 200 °C for 3 minutes. The MS recorded full scans of 20-345 amu with electron ionization at 70 eV. GC-MS data were analysed with MSD ChemStation Data Analysis software (Agilent Technologies; version D.02.00.275) and AMDIS (Geithersburg, MD; version 2.66). Tentative compound identification was done by comparing mass spectra of detected compounds with those of mass spectral libraries NIST 08 combined with Wiley 9, and GC retention values of target compounds were compared to literature values measured with similar GC parameters. After removing MEA-borne volatiles, three *C. globosum*-derived compounds were verified as 3-octanone, 3-octyl acetate, and 3-methyl-1-butanol, by comparing GC retention values with those of authentic standards. For this purpose Van den Dool and Kratz retention indices were calculated from retention times of target compounds and retention times of n-alkanes (alkane standard solution series C8-C20) by using the Van den Dool and Kratz equation:

$I_x = 100n + 100(t_x - t_n)/(t_{n+1} - t_n)$ (t_x: retention time of target compound; t_n, t_{n+1}: retention times of two consecutive n-alkanes eluted immediately before and after t_x). Quantification of *C. globosum*-derived compounds was done by using manually integrated peak areas of compound characteristic ions and corresponding calibration curves for authentic compounds diluted with paraffin (3-octanone: 96 %, CAS: 106-68-3, Merck, Darmstadt, Germany; 3-octyl acetate: 98 %, CAS: 4864-61-3, Sigma-Aldrich, St. Gallen, Switzerland; 3-methyl-1-butanol: %, CAS: 123-51-3, Sigma-Aldrich) (Appendix: Figure A.2.1). Headspace concentrations (ng/cm^3) were calculated from peak areas and Antoine coefficients (component specific constants) by means of the Antoine equation (Yaws 2007).

2.3.3 SELECTION OF VARIABLES FOR CHARACTERISATION OF COLLEMBOLA RESPONSES

Three experiments were conducted to assess responses of Collembola with respect to the searching and the contact phase of food selection (Table 2.1) to test whether wounding of fungal tissue affects the foraging behaviour (food-finding and feeding decision) of Collembola. The selection of suitable experimental setups and variables based on preliminary experiments and direct behavioural observation.

Table 2.1: *Subject of the present study and overview on components of the food selection process (adapted from* Dethier et al. *(1960),* Schoonhoven et al. *(2005)). By assuming that* C. globosum *colonies emit volatiles that Collembola can use as cues for food selection, the volatile designations (third column) refer to behavioural responses of Collembola characterised by the measured variables (second column).*

Food-selection phase	*Variables measured*	*Volatile designation*	*Null hypothesis*
Searching (off-patch) Location of potential fungal food sources	(1) Latency to the first arrival at the 'central zone'	Attractant	Collembola movement patterns are not affected by wound-activated volatiles or other wounding-related cues of *C. globosum*
		Repellent	
	(2) Percentage of time in the 'central zone' (3) Mean duration of 'central zone' visits (4) Duration of the first 'central zone' visit	Arrestant	
	(5) Number of 'substrate biting' events	Phagostimulant (acceptance)	The C8-compound 3-octanone has no effect on the 'substrate biting' activity of collembola
Contact (on-patch) Evaluation of potential fungal food souces	(6) Latency to the first colony contact (7) Latency to the first feeding event (8) Duration of the first feeding event (9) Latency to onset of feeding during the first contact with the fungal colony (10) Number of colony contacts prior to the first feeding event (11) Contact time (12) Feeding time (13) Mean duration of colony contacts (14) Mean duration of feeding events	Deterrent (rejection)	Contact behaviour and acceptance of fungal colonies as food source is not affected by wounding-dependent changes in fungal chemical cues

VARIABLES WITH RESPECT TO THE SEARCHING PHASE OF FOOD SELECTION

The semi-automated tracking platform EthoVision® XT (version 8.0; Noldus Information Technology, Wageningen, Netherlands) (Noldus *et al.* 2001) was used to analyse movement patterns of Collembola in a no-choice olfactometer in the presence of unwounded and wounded *C. globosum* colonies without direct contact to the colonies. To reveal differences in the attractivity of respective colonies, in EthoVision® XT the option 'arena settings' was employed

to define a 'central zone' around the volatile source and by means of spatial measurements isopod movement was assessed in relation to it. Based on preliminary experimental observation of Collembola responses to a known attractive food source (dried baker's yeast) and yeast-free controls (Appendix: Figure A.2.3) the following variables were selected to reveal volatile-mediated attraction and arrestance: 'latency to the first arrival at the central zone', 'duration of the first central zone visit', 'residence time in the central zone', and 'mean duration of central zone visits' (Table 2.1). If Collembola are more strongly attracted to wounded colonies compared to unwounded colonies they are expected to arrive more rapidly at the 'central zone'. If wound-activated changes in the fungal volatile profile additionally or exclusively cause arrestance, Collembola are expected to reside longer in the 'central zone' of wounded colonies.

VARIABLES WITH RESPECT TO THE CONTACT PHASE OF FOOD SELECTION

To test whether wounded *C. globosum* colonies are more accepted as food source than unwounded colonies, the contact behaviour of Collembola was observed. Following information on the behaviour of Collembola was obtained from video recordings using the computer software The Observer® XT (version 10.0, Noldus Information Technology BV, Wageningen, The Netherlands) (Noldus 1991): number of observations with feeding event, contact and feeding time, mean duration of colony contacts and feeding events, latency to the first colony contact and first feeding event, feeding onset latency after the first colony contact, duration of the first feeding event, and number of colony contacts prior to the first feeding event (Table 2.1). Contact to the resource patch was assumed when the Collembola's body or just an antenna overlapped with the patch. Feeding was assumed when Collembola remained stationary whilst the head was moved up and down rhythmically and regularly, and the Collembola's body made short forth and back twitches. If wounded colonies are more accepted than unwounded, Collembola are expected to get in contact and start feeding faster, and spend more time in contact and/or with feeding. Higher acceptance is further assumed if Collembola are arrested at the food source after the first contact, viz. if the duration of the first feeding event is prolonged and the mean duration of colony contacts and/or feeding events is higher.

'SUBSTRATE BITING' BEHAVIOUR

Preliminary direct observation of the behaviour of Collembola revealed that *F. candida*, *S. curviseta*, *H. nitidus*, and *Tomocerus vulgaris* made feeding characteristic movements in a food-free environment, designated as 'substrate biting', while leaving biting marks on the water agar surface (Figure 2.5). In the presence of a *C. globosum* colony Collembola left more biting marks the closer they came to the fungus (Figure 2.1a). Furthermore, Collembola primarily moved through and fed on wounded parts of the colony (Figure 2.1b). Based on these observations, I suppose that wound-activated volatiles function as phagostimulants for Collembola and 'substrate biting' may be considered as substrate probing ('test-biting') which is performed by Collembola in the presence of fungal volatiles to locate a fungal food source (Table 2.1). Thus, I hypothesise that wound-activated volatiles of *C. globosum* induce 'substrate biting' behaviour in Collembola. As wounding of *C. globosum* fungal tissue caused a significant increase in 3-octanone concentrations (Section 2.4), this C8-compound was tested for triggering 'substrate biting' behaviour (Table 2.1).

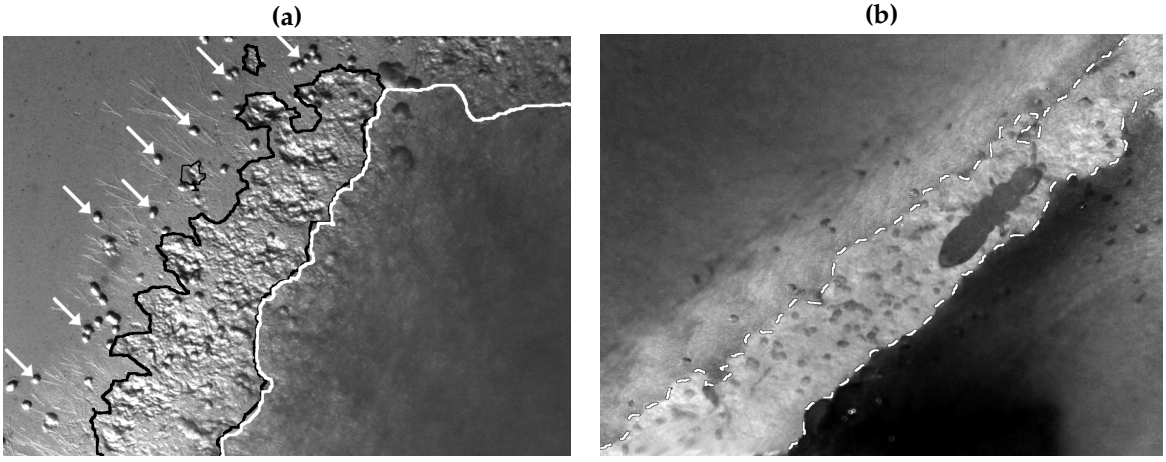


Figure 2.1: *Macro photographs of* F. candida *feeding/biting patterns in the presence of* ***(a)*** *unwounded and* ***(b)*** *wounded* C. globosum. ***(a)*** *The number of biting marks (outlined in black) and fecal pellets (white arrows) increases the closer a Collembola comes to the fungal colony (outlined in white).* ***(b)*** *A Collembola preferably moves along a corridor (indicated by the dashed white line; formed by artificial wounding with a wire loop) through the fungal tissue, leaving feeding traces and fecal pellets. Collembola preferably feed on wounded parts of* C. globosum *tissue and, even in absence of a fungal colony, Collembola show 'substrate biting' behaviour. These preliminary observations led to the hypothesis that wounding-dependent volatiles trigger 'substrate biting' in Collembola.*

2.3.4 EXPERIMENTAL SETUP

TRACKING OF COLLEMBOLA MOVEMENT (OFF-PATCH)

The movement of individual *F. candida*, *S. curviseta*, and *H. nitidus* was recorded in darkness at 20°C in response to unwounded and wounded *C. globosum* colonies by using a no-choice still air olfactometer and the computer software CyberLink PowerDirector® (CyberLink Corporation, Taipei, Taiwan). *C. globosum* fungal colonies were grown for five days in sterilized circular polypropylene pots (7 mm in hight, 25 mm in diameter) filled with 3 ml MEA. Glass Petri dishes (10.1 cm in diameter), each filled with 45 ml water agar, were used as experimental arenas (Figure 2.2a). The water agar provided a smooth and moist surface, on which Collembola could easily move, and which protected animals from desiccation. According to Auclerc *et al.* (2010), food-derived volatiles only become relevant when Collembola forage in close distance (25 mm) to the volatile source. This aspect was considered in the specification of the arena dimensions. The dimensions allowed the animals to move in a maximum distance of 36 mm and minimum distance of approximately 7 mm (height of the sample vessel) to the volatile source. The experimental setup consisted of an infrared camera focused on the circular arena, which was illuminated by two infrared lamps (Kema Electronic Co., 12-15 V/DC, M120) installed below. To ensure indirect and even illumination the lamps were placed within a polystyrene box covered with a frosted perspex screen (Figure 2.2b). Immediately before the start of recording a fresh unwounded or wounded fungal colony was placed in the centre of the arena and, thereupon, the arena was covered with a glass plate. A single animal was carefully released into the experimental arena through a glass tube (5 mm in diameter). Before starting the 15-minute recording session the animal remained trapped in the glass tube for 2 minutes for acclimatisation. The smooth surface of the polypropylene pots containing the colonies prevented animals from entering or touching the fungal colony. In EthoVision® XT the position of the animal was determined every 0.52 seconds. With 20 replicates per treatment and an equal number observations per treatment and day (randomised order) this experiment was conducted over several days.

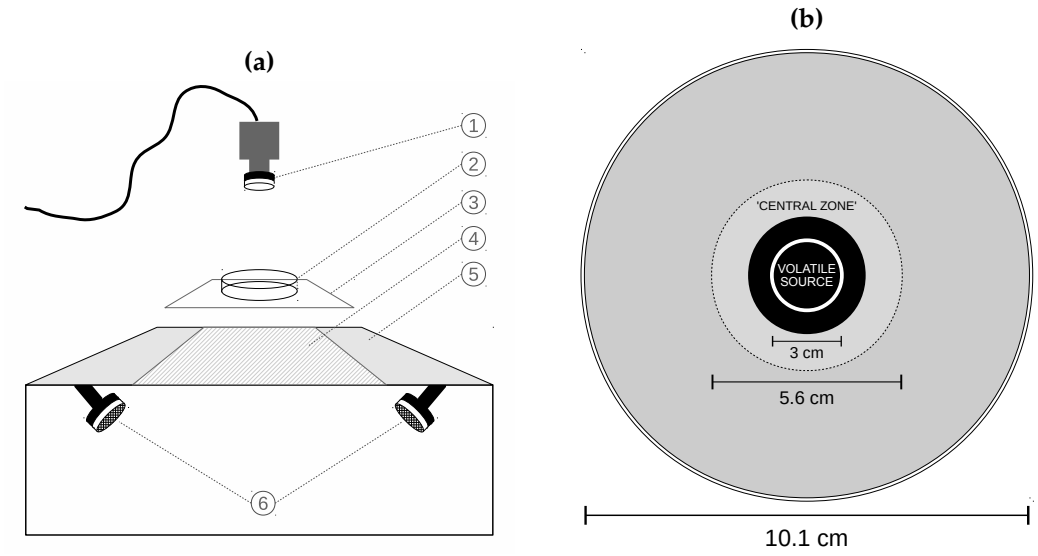


Figure 2.2: *Experimental setup for observation of Collembola movement.* ***(a)*** *Dimensions of the experimental arena with the 'central zone' defined around the volatile source. To avoid background noise and disruptions of the automated tracking process, in EthoVision® XT the black area enclosing the volatile source was subtracted from the arena.****(b)*** *Tracking device; (1) infrared camera, (2) holding device for installation of the experimental arena, (3) arena holder (perspex), (4) frosted perspex screen for even illumination, (5) polystyrene box, (6) infrared lamps (indirect illumination via light reflection).*

OBSERVATION OF COLLEMBOLA CONTACT BEHAVIOUR (ON-PATCH)

The on-patch behaviour of *F. candida*, *S. curviseta*, and *H. nitidus* Collembola in the presence of unwounded and wounded colonies of *C. globosum* was investigated by direct observation via video recordings. This experiment was conducted over several days with an equal number of recordings per treatment and day (randomised order, n=20). A Canon EOS 60D reflex camera coupled to a Zeiss Discovery V8 stereo microscope by a macro lens (17-70 mm/F2.8-4.5) was used for recording. Experimental arenas were prepared as follows: polystyrol Petri dishes (10 mm in height, 35 mm in diameter) were filled with 2 ml water agar and provided with a hole (4 mm in diameter) in the centre. The holes were filled with 20 μl malt extract agar and, after hardening, fungal tissue was transferred to the medium. Fungal colonies were directly incubated in experimental arenas for four days. The wounding treatment was done immediately before starting a recording and subsequently, a single animal was released at the margin of the arena.

QUANTIFICATION OF COLLEMBOLA 'SUBSTRATE BITING' BEHAVIOUR

F. candida and *S. curviseta* Collembola were exposed to the authentic compound 3-octanone in concentrations of w/w 10^{-2}-10^{-4} (diluted with pentane) and pure pentane served as solvent control. Standard 24-well plates (15.5 mm well diameter) were filled with sterilized water agar (0.5 ml/well) and, after hardening, 10 µl of compound solution were pipetted on the agar surface of each well. After evaporation of the solvent a single animal was added to each well. The plates were covered with fine gauze and a lid to prevent animals from escaping. After 30 minutes, the animals were carefully removed and the number of wells with bite marks was counted by means of a stereo microscope. Wells with bite marks on the agar surface were rated with value '1' and wells without marks with '0' to obtain a binary data structure.

2.3.5 STATISTICS

All statistical analysis were conducted using the R statistical environment (RStudio, version 1.0.153 for Mac OS X, R Development Core Team (2008)). Statistical tests regarding behavioural responses were individually applied on the three different Collembola species *F. candida*, *S. curviseta*, and *H. nitidus*, i.e. differences in behavioural responses between species were not tested to avoid bias caused by differences in species-specific physical properties.

Linearity of calibration curves used for determination of headspace concentrations of individual volatile compounds was tested by means of a linear model. Headspace concentrations (ng/cm^3) of 3-octanone, 3-octyl acetate, and 3-methyl-1-butanol were calculated from model estimates (slope, intercept) of calibration plots (Appendix: Figure A.2.1). Treatments (*C. globosum* unwounded, wounded) were compared using the non-parametric Wilcoxon-Mann-Whitney test with exact null distribution (α=0.05, distribution='exact', function 'wilcox_test', package 'coin' in R), individually applied on each volatile compound, since original as well as log-transformed data did not match the normal distribution pattern.

The Wilcoxon-Mann-Whitney test was also applied to test whether the 'residence time in the central zone' and the 'mean duration of central zone visits' differ between treatments (unwounded, wounded) (searching phase). With respect to the contact phase, the effect of wounding on the 'contact time' with fungal colonies, 'feeding time', 'mean duration of

colony contacts', 'mean duration of feeding events', and the 'colony contact frequency prior to the first feeding event' in the presence of unwounded and wounded fungal colonies, was also analysed using Wilcoxon-Mann-Whitney tests. Observations without a 'central zone' visit, contact and/or feeding event were excluded from the calculation of the 'mean duration of central zone visits', the 'mean duration of colony contacts', and the 'mean duration of feeding events', respectively. Effect size is given by Cohen's d (difference between two means divided by a standard deviation) (Cohen 1988) in case of same sample size, and Hedge's g in case of different sample size.

Time-dependent event data obtained from the searching phase ('latency to the first arrival at the central zone', 'duration of the first central zone visit') and the contact phase ('latency to the first contact', 'latency to the first feeding event', 'duration of the first colony contact', 'duration of the first feeding event', 'feeding onset latency after the first colony contact') in response to unwounded and wounded *C. globosum* colonies, were analysed using Cox regression models (function 'coxph', package 'survival' in R) based on likelihood estimates. With respect to the 'duration of the first central zone visit', the 'duration of the first colony contact', the 'duration of the first feeding event', and the 'feeding onset latency after the first colony contact', durations that exceeded the observation time were censored and rated as '0' in the 'status' argument of a Cox model. Prior to the application of a Cox regression model the global as well as per-variable proportionality of hazards was tested by goodness-of-fit $\tilde{\chi}^2$ tests (function 'cox.zph' in R) and visually examined by means of partial residuals plots as proposed by Schoenfeld (1980; 1982). The effect size is represented by the hazard ratio ($\exp(coef)$). As the hazard rate is defined as the instantaneous risk for the occurrence of an event within a defined observation period for a particular group, the hazard ratio represents the difference between hazard rates of treatment groups.

The effect of treatment (unwounded, wounded) on the total number of feeding events (binary data) was tested using a generalized linear model with binomial error distribution and 'logit' link function (function 'glm' in R). A generalized linear model with binomial distribution and 'logit' link function was also applied on the binary data set obtained in the course of the 'substrate biting' assay to test whether the number of 'substrate biting' events of *F. candida* and *S. curviseta* differ between 3-octanone concentrations w/w 10^{-4} and 10^{-3}, and pentane solvent controls. The explanatory variable '3-octanone concentration' entered the model as a continuous variable (ranging from 0 to 10^{-3}). Integration of the second order term of '3-octanone concentration' didn't improve the model and was excluded.

2.4 RESULTS

2.4.1 VOLATILE PROFILES OF UNWOUNDED AND WOUNDED *C. globosum* COLONIES

The headspace SPME-GC-MS analysis of volatile profiles of *C. globosum* grown on MEA resulted in the detection of 18 compounds. Whereas 11 compounds were detected in *C. globosum* as well as MEA control samples, 4 compounds were exclusively present in MEA control samples, and three compounds could be found to be produced by *C. globosum* (Appendix: compound list Table A.2.2; chromatograms Figure A.2.2). Fungal volatiles were identified as 3-octanone, 3-octyl acetate, and 3-methyl-1-butanol. Compound concentrations were significantly increased in response to the wounding treatment concerning 3-octanone (Wilcoxon-Mann-Whitney test, z=-3.36, p<0.001, Cohen's d=12.42, CI: 7.59, 17.25) and 3-octyl acetate (Wilcoxon-Mann-Whitney test, z=-2.21, p=0.028, Cohen's d=1.68, CI: 0.43, 2.93) (Figure 2.3a, 2.3b). Concentrations of 3-methyl-1-butanol were not affected by wounding (Wilcoxon-Mann-Whitney test, z=0.53, p=0.645, Cohen's d=0.47, CI:-0.62, 1.55) (Figure 2.3c).

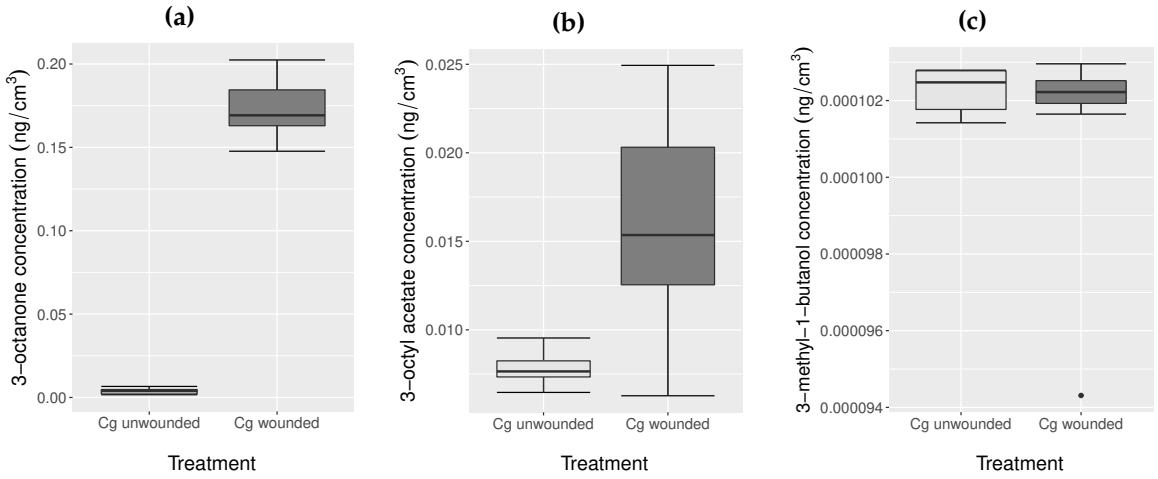


Figure 2.3: *Headspace concentrations (ng/cm^3) of volatile compounds* ***(a)*** *3-octanone,* ***(b)*** *3-octyl acetate, and* ***(c)*** *3-methyl-1-butanol identified from unwounded (Cg unwounded, n=8) and wounded (Cg wounded, n=8)* C. globosum *colonies.*

2.4.2 SEARCHING PHASE OF FOOD SELECTION

The tendency of *F. candida*, *S. curviseta*, and *H. nitidus* to arrive at the 'central zone' and the 'duration of the first central zone visit' as well as the 'residence time in the central zone' were not affected by the tissue wounding treatment (Table 2.2; Appendix: Figure A.2.4, A.2.5, A.2.6, A.2.7). These results allow the acceptance of the null hypothesis and indicate that there were no differences in volatile-mediated attraction or arrestance of Collembola between the volatile sources (unwounded and wounded *C. globosum* colonies).

Table 2.2: *Searching phase: test statistic for variables 'latency to the first arrival at the central zone' (arrival tendency), 'duration of the first central zone visit' (departure tendency) (Cox regression), 'residence time in the central zone', and 'mean duration of central zone visits' (Exact Wilcoxon-Mann-Whitney test). For Cox regression models the $\tilde{\chi}^2$ value and for Wilcoxon tests the Z-score is given.*

Species	*Variable*	N	*Events*	Df	$\tilde{\chi}^2/Z$	$\exp(coef)^{(*)}$	$P-value$
F. candida	First arrival	40	29	1	0.13	1.14	0.723
H. nitidus	First arrival	40	39	1	0.03	1.06	0.859
S. curviseta	First arrival	40	36	1	0.03	1.06	0.854
F. candida	Duration first visit	29	27	1	0.24	0.83	0.624
H. nitidus	Duration first visit	39	37	1	0.93	0.71	0.335
S. curviseta	Duration first visit	36	33	1	0.07	1.10	0.790
F. candida	Time in zone	40		1	1.10		0.276
H. nitidus	Time in zone	40		1	-0.81		0.925
S. curviseta	Time in zone	40		1	0.69		0.499
F. candida	Mean duration	29		1	0.71		0.747
H. nitidus	Mean duration	39		1	0.17		0.879
S. curviseta	Mean duration	36		1	-0.36		0.731

$^{(*)}$ Ratio of hazard rates between treatment groups (unwounded vs. wounded *C. globosum* colonies). Given hazard ratios relate to the wounded treatment group

2.4.3 CONTACT PHASE OF FOOD SELECTION

In general, the contact behaviour of the three tested Collembola species was influenced by fungal tissue wounding, however, the effect was not consistent across all tested variables (Table 2.3, 2.4). Whereas the number of observations with feeding event was not affected by fungal treatment in *F. candida* (deviance=2.18, df=1, p=0.140) and *S. curviseta* (deviance=0, df=1, p=0.100), significantly more *H. nitidus* individuals exhibited feeding on wounded colonies compared to unwounded colonies (deviance=5.13, df=1, p=0.023) (Appendix: Figure A.2.8b). *H. nitidus* tendency to start feeding on wounded colonies was 3.41 times higher

compared to unwounded colonies (Table 2.4, Appendix: Figure A.2.15b). Moreover, *H. nitidus* individuals spent a significantly higher proportion of the observation time at wounded colonies and fed significantly longer on these colonies compared to unwounded colonies (Table 2.3, Appendix: Figure A.2.10b, A.2.12b). The duration of both colony visits and feeding events at wounded colonies was significantly longer compared to unwounded colonies (Appendix: Figure A.2.11b, A.2.13b). Similar to *H. nitidus*, the tendency of *F. candida* individuals to start feeding on a wounded *C. globosum* colony was 2.08 times higher compared to unwounded colonies (Table 2.4, Appendix: Figure A.2.15a). The duration of colony visits was also significantly longer in the wounding treatment group (Table 2.3, Appendix: Figure A.2.11a). Once *F. candida* contacted a colony, onset of feeding was 2.47 times faster in the presence of a wounded colony compared to unwounded colonies (Table 2.4, Appendix: Figure A.2.16a). With respect to *S. curviseta*, exclusively the colony 'contact frequency prior to the first feeding event' was significantly affected by the treatment (Table 2.3). Individuals less frequently visited wounded colonies prior to feeding compared to unwounded colonies (Appendix: Figure A.2.9c). Contrary to expectations, with inverted hazard ratios ($1/\exp(coef)$) of 2.44 and 2.22 the tendency of *S. curviseta* and *F. candida* Collembola to contact an unwounded *C. globosum* colony was more than twice as high as the tendency to contact a wounded colony (Table 2.4, Appendix: Figure A.2.14a, A.2.14c). Further contrasting results, viz. wounding-related negative effects on Collembola acceptance, were not found.

Table 2.3: *Contact phase: Wilcoxon-Mann-Whitney test statistic of variables: 'colony contact frequency prior to the first feeding event', 'contact time', 'mean duration of colony contacts', 'feeding time', 'mean duration of feeding events'. Effect size is represented by Cohen's d in case of equal sample size and by Hedge's g in case of different sample size, with confidence interval in parentheses.*

Species	*Variable*	*N*	*Df*	*Z*	*Effect size*	*P − value*	(*)
F. candida	Contacts prior feeding	30	1	1.83	0.49 (-0.28, 1.26)	0.070	
H. nitidus	Contacts prior feeding	17	1	0.67	0.39 (-0.76, 1.52)	0.532	
S. curviseta	Contacts prior feeding	26	1	2.85	1.05 (0.19, 1.91)	**0.004**	▽
F. candida	Contact time	40	1	-1.65	-0.40 (-1.05, 0.24)	0.102	
H. nitidus	Contact time	40	1	-2.06	-0.77 (-1.43, -0.11)	**0.040**	▲
S. curviseta	Contact time	40	1	1.10	0.49 (-0.16, 1.14)	0.280	
F. candida	Mean contact	40	1	-2.03	-0.29 (-0.93, 0.35)	**0.043**	▲
H. nitidus	Mean contact	35	1	-2.34	-0.72 (-1.43, -0.01)	**0.019**	▲
S. curviseta	Mean contact	38	1	-0.64	-0.47 (-1.14, 0.20)	0.534	
F. candida	Feeding time	40	1	-1.92	-0.55 (-1.21, 0.10)	0.055	
H. nitidus	Feeding time	40	1	-2.46	-0.76 (-1.42, -0.09)	**0.013**	▲
S. curviseta	Feeding time	40	1	0.48	0.34 (-0.31, 0.98)	0.637	
F. candida	Mean feeding	30	1	-0.73	-0.24 (-1.00, 0.52)	0.483	
H. nitidus	Mean feeding	17	1	-2.21	-0.68 (-1.84, 0.48)	**0.027**	▲
S. curviseta	Mean feeding	26	1	0.74	0.40 (-0,42, 1.22)	0.479	

(*) With regard to the respective variable, ▽ indicates a significant decrease and ▲ a significant increase in the wounded treatment group in comparison to the unwounded treatment group.

Table 2.4: *Contact phase: Cox regression test statistic for time dependent event data. Variables: 'latency to the first colony contact', 'latency to the first feeding event', 'duration of the first feeding event', and 'feeding onset latency after the first contact'.*

Species	*Variable*	*N*	*Events*	*Df*	$\tilde{\chi}^2$	$\exp(coef)^{(*)}$	*P − value*	(**)
F. candida	First colony entering	40	40	1	5.32	0.45	**0.022**	▽
H. nitidus	First colony entering	40	35	1	0.04	1.07	0.849	
S. curviseta	First colony entering	40	38	1	6.03	0.41	**0.014**	▽
F. candida	First feeding	40	30	1	3.93	2.08	**0.047**	▲
H. nitidus	First feeding	40	17	1	5.91	3.41	**0.022**	▲
S. curviseta	First feeding	40	26	1	0.16	0.86	0.692	
F. candida	Duration first feeding	30	28	1	0.74	0.72	0.391	
H. nitidus	Duration first feeding	17	16	1	2.46	0.37	0.117	
S. curviseta	Duration first feeding	26	26	1	0.53	1.36	0.465	
F. candida	Feeding onset latency	30	30	1	5.35	2.47	**0.021**	▲
H. nitidus	Feeding onset latency	17	17	1	1.25	1.86	0.263	
S. curviseta	Feeding onset latency	26	26	1	0.57	1.36	0.452	

(*) Ratio of hazard rates between treatment groups (unwounded vs. wounded *C. globosum* colonies). Given hazard ratios relate to the wounded treatment group, i.e. values lower than 1 indicate decreased hazard and values higher than 1 indicate increased hazard in the wounded treatment group compared to the unwounded treatment group.

(**) With regard to the respective variable, ▽ indicates a significantly decreased hazard and ▲ a significantly increased hazard in the wounded treatment group in comparison to the unwounded treatment group.

2.4.4 'SUBSTRATE BITING' BEHAVIOUR OF *F. candida* AND *S. curviseta*

The 'substrate biting' behaviour of Collembola (total number of 'substrate biting' events) was significantly influenced by the presence of 3-octanone (Binomial regression, *F. candida*: intercept=-0.71, slope=829.94, z=3.47, p<0.001; *S. curviseta*: intercept=-0.54, slope=477.11, z=1.98, p=0.047) with an increase in biting events with increasing 3-octanone concentration (Figure 2.4). In the course of this experiment it turned out that Collembola were paralysed by 3-octanone at a concentration of w/w 10^{-2}, consequently, this concentration was excluded from the statistical analysis.

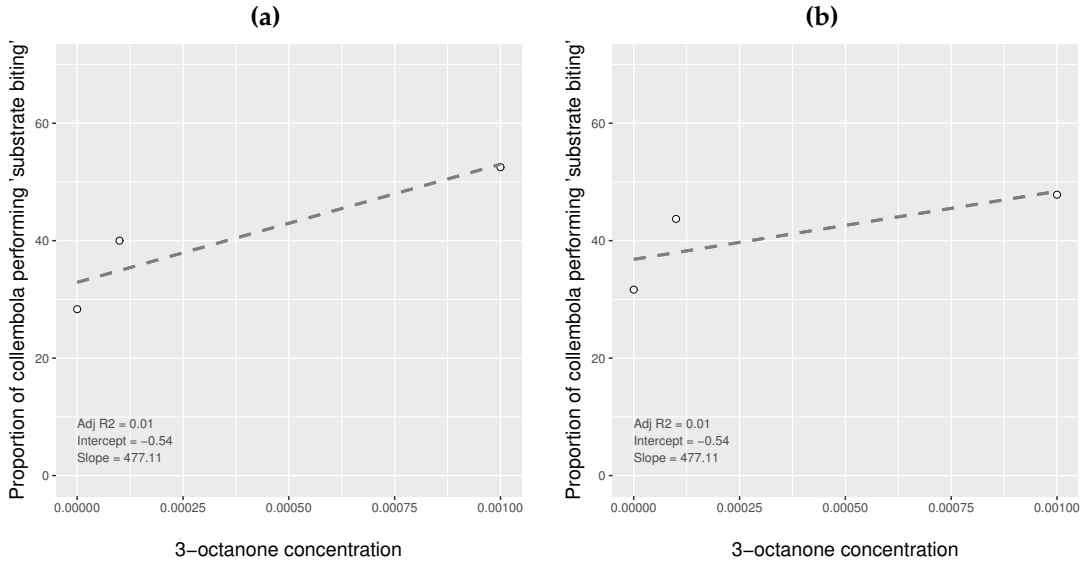
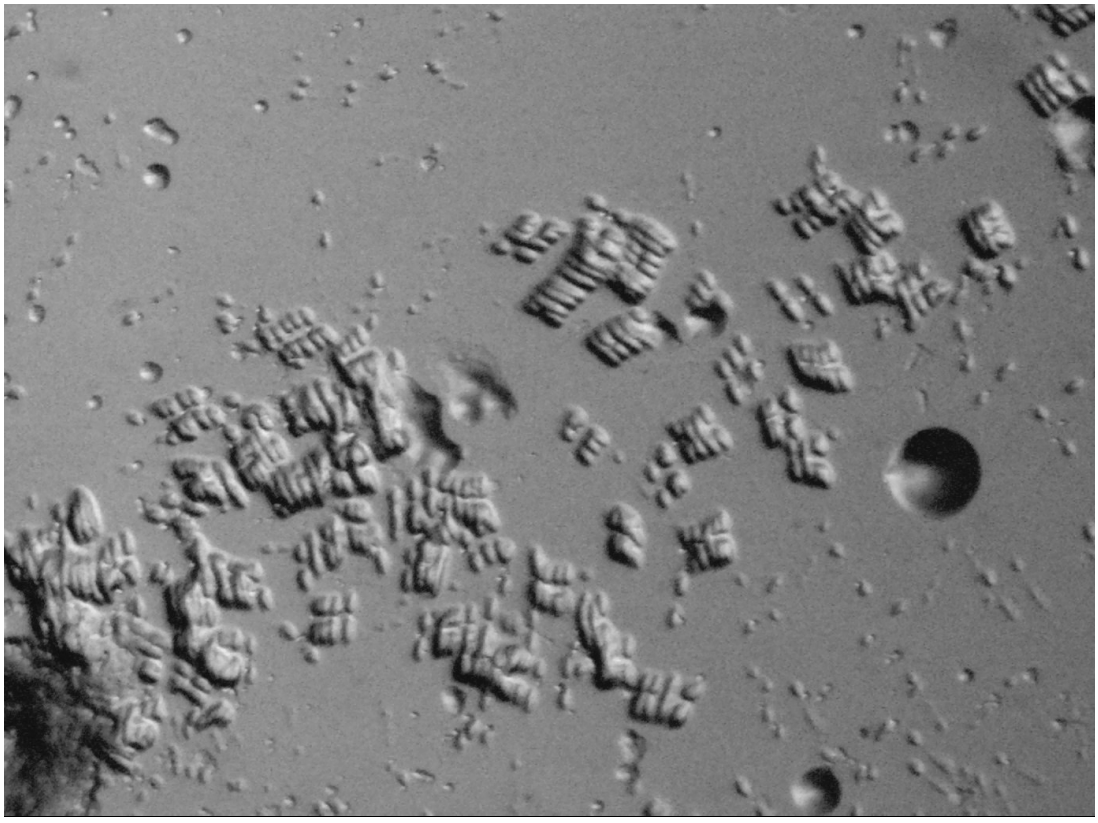


Figure 2.4: *Number of 'substrate biting' events of* ***(a)*** F. candida *and* ***(b)*** S. curviseta *in response to 3-octanone concentrations (w/w 10^{-4}, 10^{-3}) and paraffin controls.* F. candida*: p<0.001 (10^{-4}: n=120, 10^{-3}: n=120, control: n=120);* S. curviseta*: p=0.047 (10^{-4}: n=119, 10^{-3}: n=115, control: n=120).*

(a)



(b)

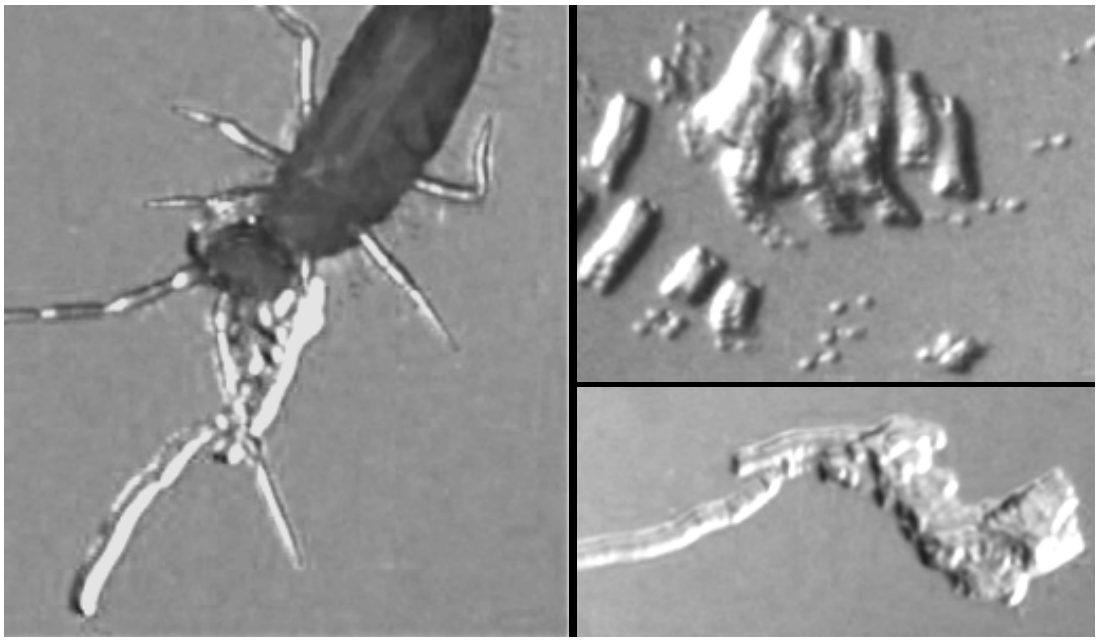


Figure 2.5: *Macro photographs of biting marks of* ***(a)*** F. candida *and* ***(b)*** S. curviseta *in the presence of 3-octanone.*

2.5 DISCUSSION

The present study, for the first time, investigated the effect of fungal tissue wounding and related changes in fungal chemistry on the foraging behaviour of facultative fungivorous Collembola by focussing on the searching phase (food location) and the contact phase (food acceptance) of foraging to get a holistic view on the food selection process (Schoonhoven *et al.* 2005). Overall, the present study confirms that fungal chemical properties, at least to some extent, influence the outcome of Collembola-fungus interactions, as it has already been proposed by earlier studies (Böllmann *et al.* 2010, Rohlfs *et al.* 2007, Stötefeld *et al.* 2012).

The headspace analysis of volatiles resulted in the identification of three compounds, 3-octanone, 3-octyl acetate, and 3-methyl-1-butanol, with increased concentrations of the two volatile oxylipins, 3-octanone and 3-octyl acetate, in wounded colonies. Whereas 3-octanone and 3-methyl-1-butanol have frequently been reported as common mushroom volatiles (e.g. Börjesson *et al.* 1992, Fiedler *et al.* 2001, Korpi *et al.* 2009), 3-octyl acetate was primarily identified from essential oils of several plant species (e.g. Akin *et al.* 2010, Handa *et al.* 1964), but also from *Penicillium* species (Matysik *et al.* 2008, Nilsson *et al.* 1996); however, its precursors, the oxylipin 3-octanol and acetic acid, are well known to be produced by different fungal species (Abraham and Berger 1994, Fiedler *et al.* 2001, Mburu *et al.* 2011, Thakeow *et al.* 2008). As 3-octyl acetate was consistently present across all samples, measured on different days, with significantly increased concentrations in wounded colonies, it can be excluded that this compound originated from a contamination. The wound-activated increase in eight-carbon oxylipin volatile compounds caused by artificial mechanical wounding of fungal tissue accords with results of other studies; similar increases were reported for *Marchantia polymorpha* (Kihara *et al.* 2014) and *Agaricus bisporus* (Combet *et al.* 2009).

Contrary to my expectation, movement patterns of the three Collembola species *F. candida*, *S. curviseta*, and *H. nitidus* were not affected by wound-activated changes in the volatile profile of *C. globosum*. This indicates that neither the blind and hemiedaphic species *F. candida* nor the hemi- and epedaphic species *S. curviseta*, and *H. nitidus* use wound-activated volatiles as information to find fungal food from distance. However, as fungi strongly differ in their wounding-specific volatile profiles, especially with respect to the activation and increase of oxylipin volatiles (Chapter 4, Table 4.3), the generality of this statement needs to be tested by including further species of filamentous fungi. Up to now, this is the first study that directly links wound-activated changes in fungi and spatial foraging behaviour of Collembola, but, other observations of arthropod responses to 3-octanone were done with

respect to different beetle species, lepidoptera, and the earthworm *E. fetida*. Whereas some beetle species were attracted to 3-octanone (Holighaus *et al.* 2014, Pierce *et al.* 1991a), the behaviour of other beetles, lepidoptera, and earthworms, was not affected by this compound (Fäldt *et al.* 1999, Pierce *et al.* 1991a, Tabata *et al.* 2011, Zirbes *et al.* 2011). Simultaneously, these studies demonstrate that behavioural responses can be different in respect of different oxylipin compounds.

In contrast to the unexpected results with respect to the searching phase of food selection, Collembola responses observed during the contact phase and the 'substrate biting' assay indicate that wound-activated changes in fungal chemistry become relevant when looking at behaviours associated with colony contact and feeding. Whereas during the searching phase only volatiles could be used as information, it is very likely that gustatory, tactile, and visual properties were used as cues for evaluation during the contact phase. The results strongly suggest that the volatile oxylipin 3-octanone functions as volatile food-finding cue and phagostimulant for Collembola and provide first evidence that at least some fungal volatile oxylipins function as cues in an analogous manner to plant volatile oxylipins (e.g. Halitschke *et al.* 2004, Mitchell and McCashin 1994, Murray *et al.* 1972, Zhang 2016). Since an increased 3-octanone concentration stimulated a higher number of Collembola to show 'substrate biting' behaviour, the higher concentration of this compound in wounded *C. globosum* colonies was also most likely responsible for the higher acceptance of these colonies as food source, primarily indicated by higher feeding tendencies. A possible scenario is that 'substrate biting' is an integral part of the foraging process and regularly exhibited by Collembola during the searching phase to permanently test if fungal food is present in immediate surroundings. If relevant fungal volatiles are present, 'substrate biting' behaviour will increase, turn to feeding, and finally lead to an increase in volatile oxylipin emissions. Presumably, the absence of volatile and/or non-volatile deterrents and presence of phagostimulants will induce continuous feeding, as it is supposed to be the mode of action in plant-herbivore systems (Schoonhoven 1968). According to Schoonhoven *et al.* (2005) the here observed 'substrate biting' behaviour can be considered as 'test-biting' (probing) and interpreted as a kind of exploratory behaviour, which is common and frequently observed in herbivorous arthropods (e.g. Chapman and Bernays 1989, Schoonhoven *et al.* 2005). As this behaviour was observed in different Collembola species it is suggested to be a fixed component of Collembola foraging behaviour. However, since only one compound was tested here, it needs to be tested whether oxylipin volatiles generally stimulate 'test-biting' and feeding in Collembola.

When focussing on the contact phase, it appears that the Collembola species *F. candida*, *S. curviseta*, and *H. nitidus*, perceive and use fungal cues for the evaluation of potential food sources differently. Whereas higher acceptance of wounded *C. globosum* colonies by *F. candida* and *H. nitidus* was reflected in different behavioural variables and most clearly shown for *H. nitidus*, only the lower number of contacts prior to the first feeding event indicated higher acceptance of these colonies by *S. curviseta*. The tendency of *F. candida* and *S. curviseta* to get in first contact with wounded colonies was even lower. This unexpected result contrasts with subsequent behavioural responses, viz. higher feeding activity and colony contact. Since Collembola were raised on a baker's yeast-based diet, a possible explanation is that wound-activated oxylipin volatiles of *C. globosum* represent unknown stimuli that initially elicited avoidance of wounded colonies by *F. candida* and *S. curviseta*.

Behavioural differences between Collembola species indicate the presence of different food-finding mechanisms and may explain observed species-specific selective feeding on (Moore *et al.* 1987, Varga *et al.* 2002) and preferences for certain fungal food sources (Hiol Hiol *et al.* 1994, Sadaka-Laulan *et al.* 1998, Thimm and Larink 1995, Tordoff *et al.* 2008, Visser and Whittaker 1977). In the field of soil biology, a long-standing question is how the great diversity of soil faunal communities could emerge, obviously, without clear niche differentiation, termed 'the enigma of soil animal diversity' (Anderson 1975). One hypothesis is that the high diversity of decomposer communities results from different exploitation of fungal resources. In fact, different soil animal species show distinct feeding preferences, and with particular respect to mites and Collembola, it was demonstrated that certain fungal species were preferred as food source (Maraun *et al.* 2003). However, selective feeding on fungi is assumed to be more strongly exhibited by epedaphically living species than by hemiedaphic species (Walsh and Bolger 1990). The finding that the epedaphic species *H. nitidus* showed the clearest response to wound-activated changes in fungal chemistry among the tested species supports this assumption. Species-specific responses to fungal volatiles may result in differential resource use, which might reduce interspecific competition and facilitate species coexistence.

Some limitations need to be specified with respect to the results of the present study. Existing studies on both plant-insect and fungus-arthropod interactions have demonstrated that behavioural responses to volatile emissions are highly variable and can be affected by synergistic (e.g. Brand *et al.* 1977, Dowd and Bartelt 1991, Mburu *et al.* 2013, Nout and Bartelt 1998) as well as concentration-dependent effects (e.g. Pierce *et al.* 1991a, Thakeow *et al.* 2008), and the type of interaction (host, non-host) (Hulcr *et al.* 2011). Different oxylipin volatiles

can provoke contrasting behavioural responses in the same species as it was shown for the fungivorous beetle *B. reticulatus* in the presence of the three oxylipin volatiles 1-octen-3-ol, 3-octanone, and 3-octanol (Holighaus *et al.* 2014). Conversely, the same compound can induce contrasting responses in different species, as it was extensively investigated with respect to the plant characteristic oxylipin volatile (*Z*)-3-hexenol (Wei and Kang 2011), but also found in fungus-arthropod interactions with respect to the common mushroom oxylipin volatile 1-octen-3-ol. This compound elicited attraction in the erotylid beetle *T. bipustulata*, the ciid beetle *S. affinis* (Drilling and Dettner 2009), and the coccinellid beetle *P. vigintimaculata* (Tabata *et al.* 2011), repelled the tenebrionid beetle *B. reticulatus* (Holighaus *et al.* 2014) and *P. minuta* Collembola (Sawahata *et al.* 2008), and functions as antifeedant for banana slugs (Wood *et al.* 2001). In the light of this variability, the foraging behaviour of fungivorous arthropods needs detailed investigation by testing the effect of further fungal (oxylipin) volatiles to assess the importance of volatiles for the emergence of different types of mutualistic and antagonistic fungus-arthropod interactions.

In conclusion, the present study represents a new approach for investigating Collembola foraging behaviour in detail by focussing on the two phases of food selection, the searching and the contact phase. Direct observation of individual Collembola responses offered novel insights on the importance of fungal volatiles in mediating fungus-arthropod interactions and Collembola food selection. Three Collembola species were found to respond differently to volatiles on different levels of the foraging process indicating the presence of species-specific foraging strategies. Moreover, this study revealed a hitherto completely overlooked behavioural component of Collembola foraging, a probing behaviour designated as 'test-biting' (Schoonhoven *et al.* 2005). With particular focus on the effect of wound-activated volatile oxylipin emissions, the common fungal oxylipin volatile 3-octanone is suggested to act as food-finding cue and phagostimulant for Collembola. Further behaviour-modifying (oxylipin) volatiles and non-volatile chemical aspects of Collembola-fungus interactions remain to be identified to fully understand the complexity of Collembola foraging decisions.

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APPENDIX

Table A.2.1: *Analytical response (TIC peak area) to authentic compounds 3-octanone (3OCT), 3-octyl acetate (3OcAc), and 3-methyl-1-butanol (IA) diluted with paraffin at four concentrations (conc) and equivalent headspace concentrations in ng/cm^3 (calibration plots: Figure A.2.1)*

$3OCT$ conc	$3OCT$ $\mathrm{ng/cm^3}$	$3OCT$ TIC peak area	$3OcAc$ conc	$3OcAc$ $\mathrm{ng/cm^3}$	$3OcAc$ TIC peak area	IA conc	IA $\mathrm{ng/cm^3}$	IA TIC peak area
0.0000001	0.0012	164135	0.0000001	0.000319	1092643	0.000000001	0.00000672	756273
0.000001	0.012	3026680	0.000001	0.00319	1927189	0.00000001	0.0000672	9065576
0.00001	0.12	58245454	0.00001	0.0319	22114012	0.0000001	0.000672	53654625
0.0001	1.2	465265131	0.0001	0.319	115522300	0.000001	0.00672	404864832

Table A.2.2: *Presence (black circle) and absence (white circle) of volatile compounds identified from headspace samples of unwounded (uw) and wounded (wo)* C. globosum *colonies (CG) and fungal-free MEA controls. Compound identities were verified by matching experimental retention indices (RI_{ex}) with literature values (RI_{Li}) and authentic compounds (RI_{Ac}). Compound numbers correspond to peak numbers in total ion current chromatograms (Figure A.2.2)*

#	Compound name	CAS	MEA control	CG unwounded	CG wounded	RI_{ex} [1]	RI_{Li} [2]	RI_{AC} [3]	Reference
1	CO2	124-38-9	●	●	●	-	-	-	-
2	N2	7727-37-9	●	●	●	-	-	-	-
3	Ethanol	64-17-5	●	●	●	479	446	-	Peng (1992)
4	Furan	110-00-9	●	●	●	502	-	-	-
5	Acetone	67-64-1	●	●	●	506	503	-	Insausti et al. (2005)
6	2-propanol	67-63-0	●	●	●	524	516	-	Xu et al. (2003)
7	1-propanol	71-23-8	●	●	●	588	595	-	Bylaite & Meyer (2006)
8	2-butanone	78-93-3	●	●	●	604	603	-	Madruga & Mottram (1998)
9	2-methylfuran	534-22-5	●	●	●	608	605	-	Methven et al. (2007)
10	5-methyl-3-heptanone	541-85-5	●	●	●	943	939	-	Rostad & Pereiran (1986)
11	Limonene	138-86-3	●	●	●	1030	1030	-	Javidnia et al. (2003)
12	2,3-butanedione	431-03-8	●	○	○	603	606	-	Mahajan et al. (2004)
13	3-methylbutanal	590-86-3	●	○	○	662	664	-	Bylaite & Meyer (2006)
14	2-methylbutanal	96-17-3	●	○	○	668	664	-	Rychlik & Bosset (2001)
15	Hexanal	66-25-1	●	○	○	803	803	-	Wu & Cadwallader (2002)
16	Isoamyl alcohol	123-51-3	○	●	●	745	750	745	Komárek (1998)
17	3-octanone	106-68-3	○	●	●	988	988	988	Javidnia (2005)
18	3-octyl acetate	4864-61-3	○	●	●	1126	1127	1126	Andriamaharavo (2014)

Van den Dool and Kratz retention indices as [1] calculated from experimental retention times [2] reported in literature (see Reference) and [3] calculated from authentic compound

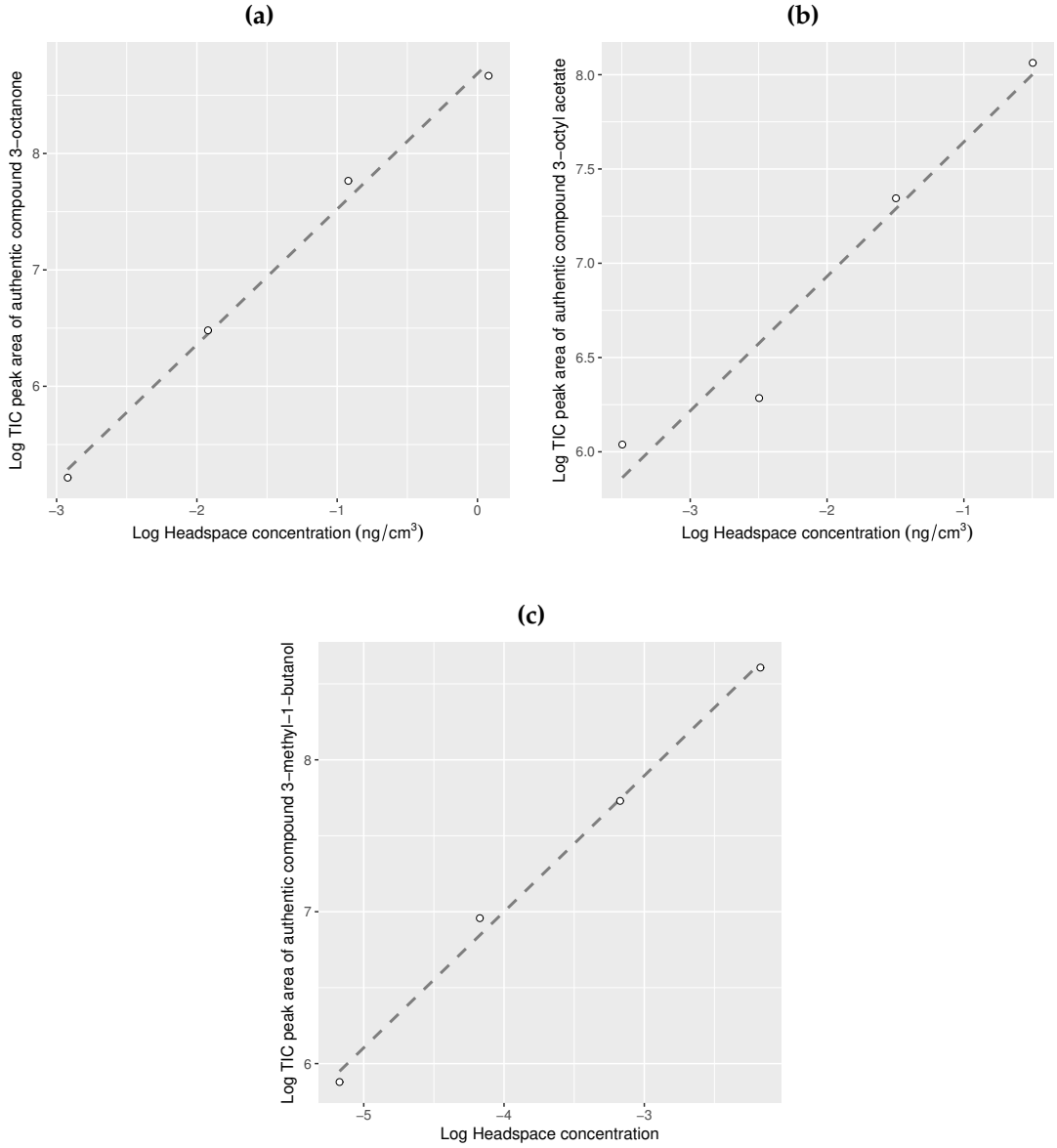

Figure A.2.1: *Logarithmic calibration plots with linear trend line (dotted, grey) representing analytical response (TIC peak area) as a function of known analyte quantity (ng/cm^3) for authentic volatile compounds* ***(a)*** *3-octanone (adjusted R^2 = 0.9999, intercept = 3067321.94, slope = 385918297.55, p = 0.0031),* ***(b)*** *3-octyl acetate (adjusted R^2 = 0.9997, intercept = 4009911.35, slope = 351864015.66, p = 0.0059), and* ***(c)*** *3-methyl-1-butanol (adjusted R^2 = 0.9998, intercept = 6131182.63, slope = 59437062504.38, p = 0.0048). Respective linear model estimates were used for quantification of headspace concentrations of* C. globosum *fungal volatiles.*

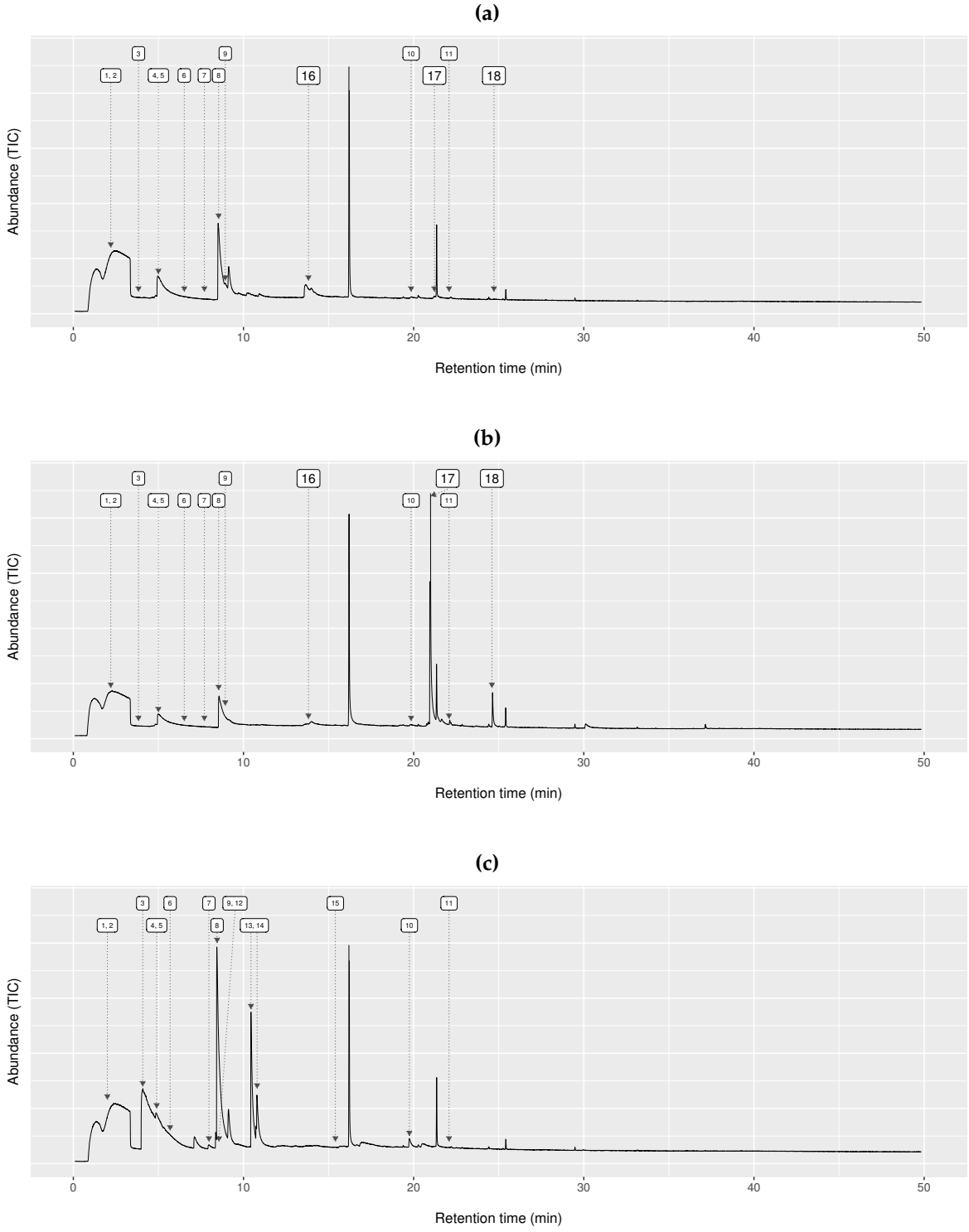

Figure A.2.2: *Total ion current chromatograms of* ***(a)*** *unwounded and* ***(b)*** *wounded* C. globosum *colonies, and* ***(c)*** *fungal-free MEA controls. Peak numbers correspond to compound numbers in Table A.2.2.*

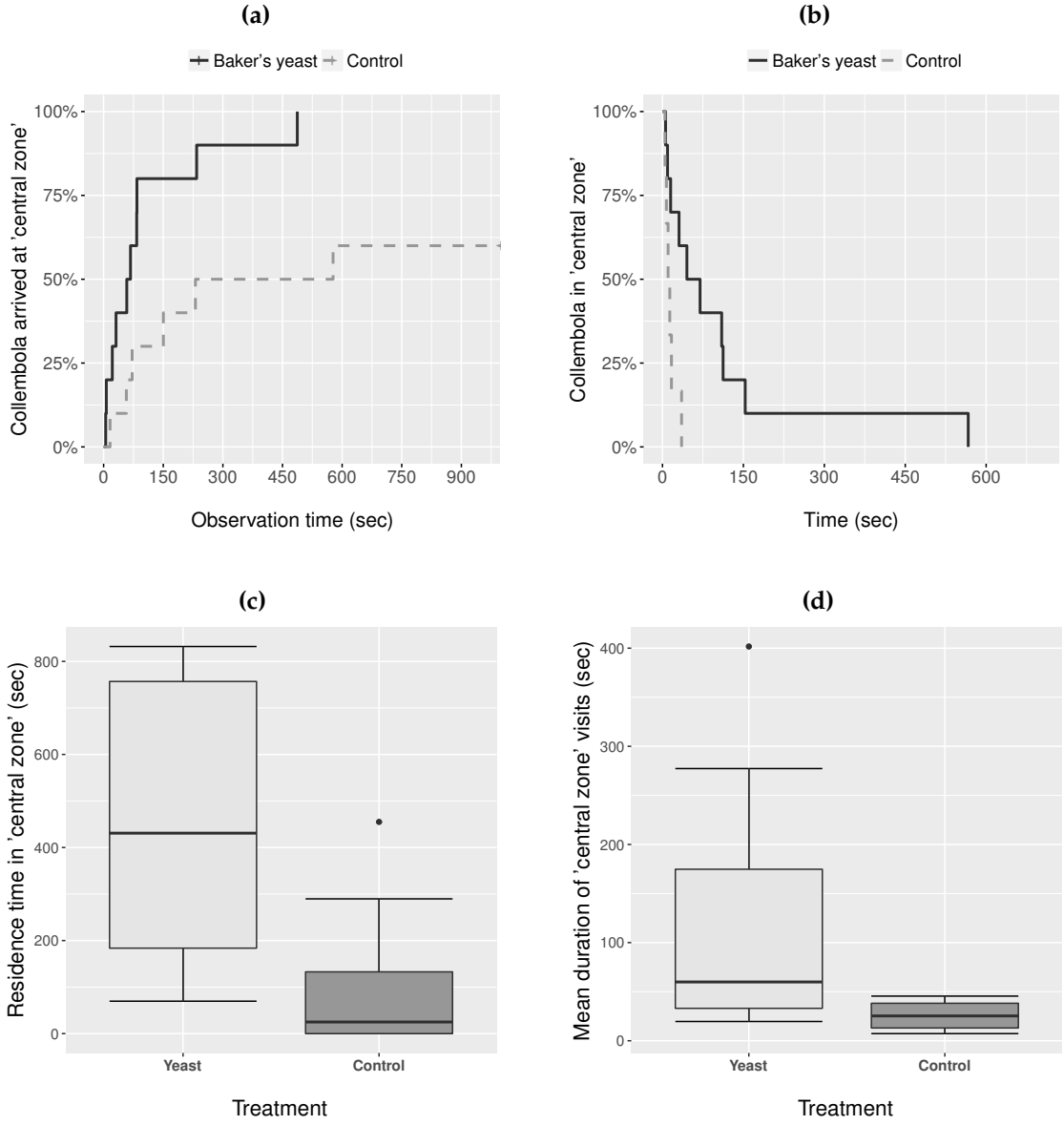

Figure A.2.3: *Verification of no-choice olfactometer arena functioning for the detection of Collembola attracting or arresting volatile sources by comparison of individual* F. candida *responses to the known attractive volatile source dried baker's yeast (applied on the surface of malt extract agar) and yeast free malt extract agar controls within an observation period of 15 minutes.* ***(a)*** *Kaplan-Meier curves for the 'latency to the first arrival at the central zone',* ***(b)*** *'duration of the first central zone visit',* ***(c)*** *'residence time in the central zone', and* ***(d)*** *'mean duration of central zone visits'. Statistical analysis validated arena functioning as arrival tendency of Collembola was significantly higher in response to baker's yeast samples compared to controls (Cox regression, likelihood ratio test: $\tilde{\chi}^2$=5.91, df=1, p=0.015, exp(coef)=3.715, N=20, arrivals=16), indicating attraction. Higher 'residence time in the central zone' (Exact Wilcoxon-Mann-Whitney test: Cohen's d=1.5, z=3.04, p=0.001), 'mean duration of central zone visits' (Exact Wilcoxon-Mann-Whitney test: Hedge's g=0.89, z=2.17, p=0.031) and prolonged first 'central zone' visit in response to baker's yeast (Cox regression, likelihood ratio test: $\tilde{\chi}^2$=5.11, df=1, p=0.024, exp(coef)=4.431, N=16, departures=16) indicate arrestance.*

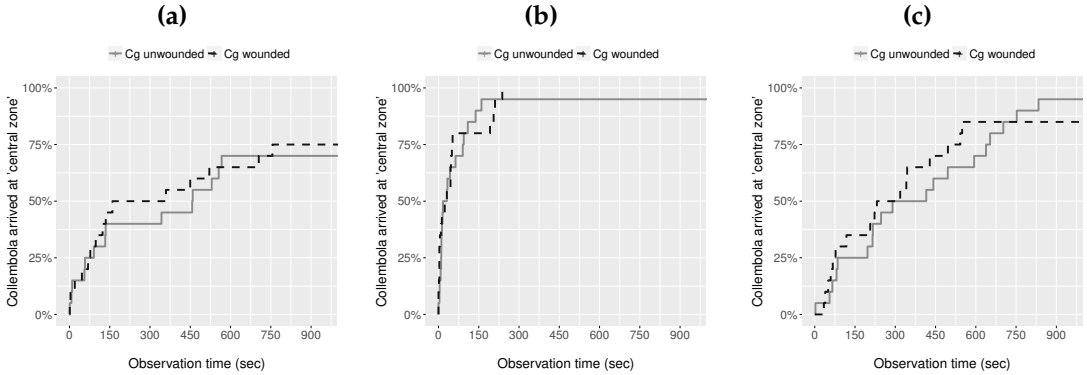

Figure A.2.4: *Searching phase: Kaplan-Meier curves for the 'latency to the first arrival at central zones' in response to unwounded and wounded* C. globosum *colonies (n=20) within an observation period of 15 minutes.* ***(a)*** F. candida*: p=0.723;* ***(b)*** H. nitidus*: p=0.859;* ***(c)*** S. curviseta*: p=0.854.*

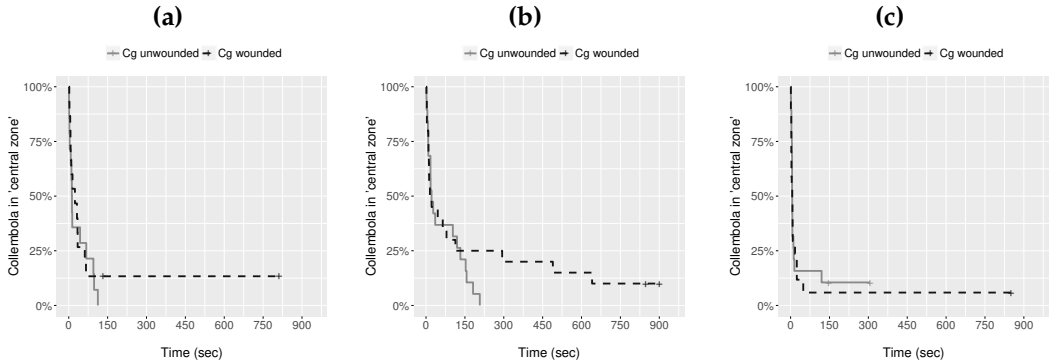

Figure A.2.5: *Searching phase: Kaplan-Meier curves for the 'duration of the first central zone visit' (departure tendency) in response to unwounded and wounded* C. globosum *colonies within an observation period of 15 minutes.* ***(a)*** F. candida*: p=0.747, N=29;* ***(b)*** H. nitidus*: p=0.879, N=39;* ***(c)*** S. curviseta*: p=0.731, N=36.*

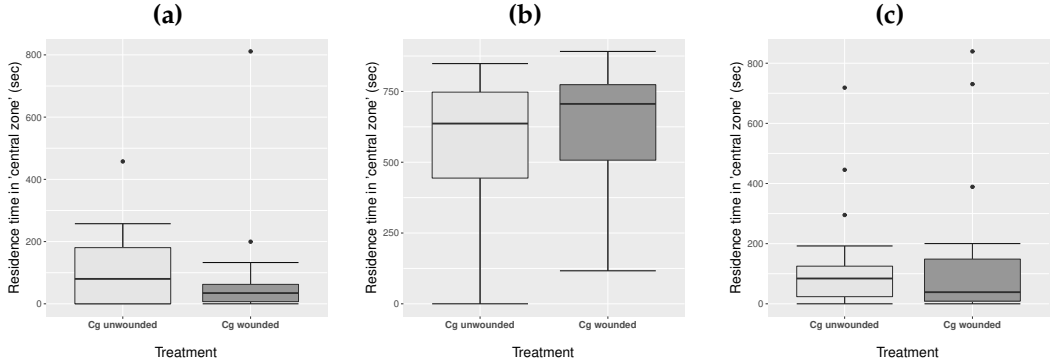

Figure A.2.6: *Searching phase: 'Residence time in the central zone' in response to unwounded and wounded* C. globosum *colonies (n=20) within an observation period of 15 minutes.* ***(a)*** F. candida*: p=0.276;* ***(b)*** H. nitidus*; p=0.925;* ***(c)*** S. curviseta*: p=0.499.*

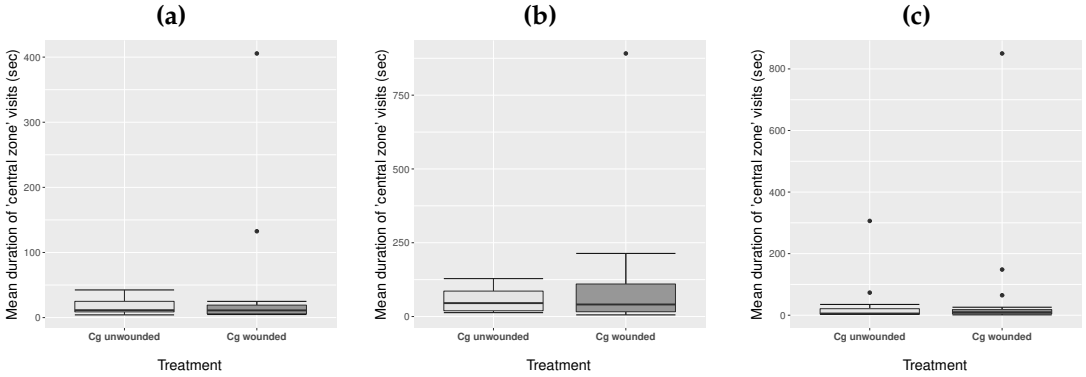

Figure A.2.7: *Searching phase: 'Mean duration of central zone visits' in response to unwounded and wounded* C. globosum *colonies within an observation period of 15 minutes.* ***(a)*** F. candida*: p=0.908, N=29;* ***(b)*** H. nitidus*: p=0.425, N=39;* ***(c)*** S. curviseta*: p=0.825, N=36.*

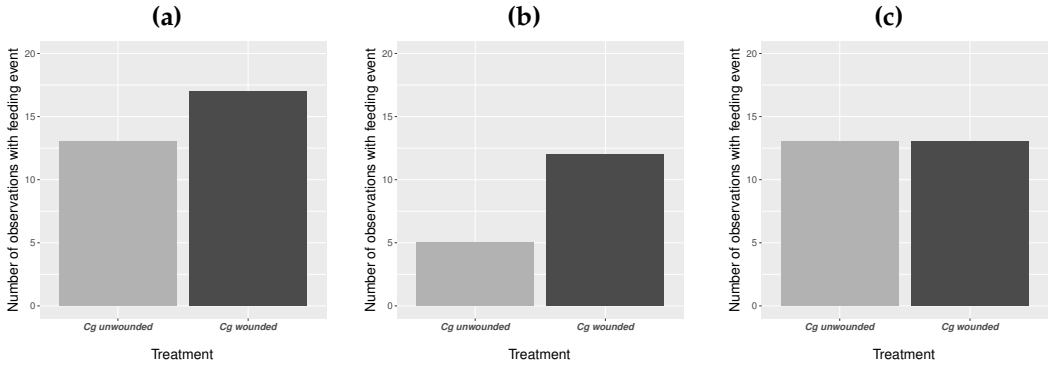

Figure A.2.8: *Contact phase: 'Total number of feeding events' in response to unwounded and wounded* C. globosum *colonies (n=20).* ***(a)*** F. candida*: p=0.140;* ***(b)*** H. nitidus*: p=0.023;* ***(c)*** S. curviseta*: p=0.1.*

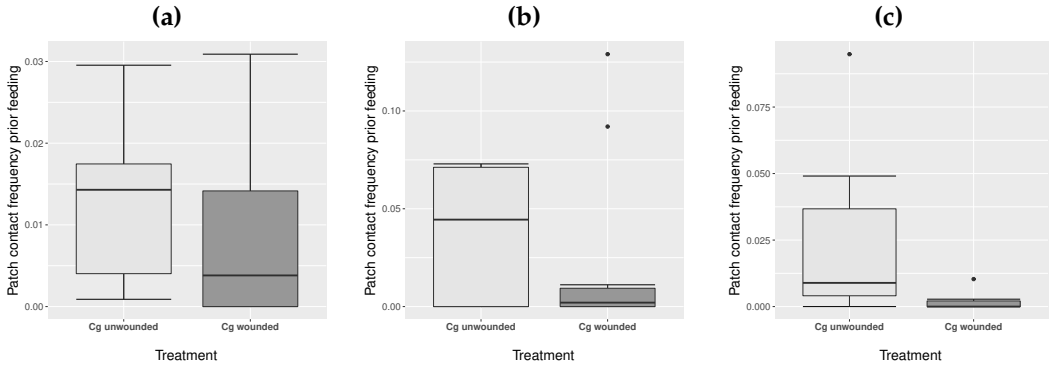

Figure A.2.9: *Contact phase: 'Colony contact frequency prior to the first feeding event' in response to unwounded and wounded* C. globosum *colonies.* ***(a)*** F. candida*: $n_{unwounded}$=13, $n_{wounded}$=17, p=0.070;* ***(b)*** H. nitidus*: $n_{unwounded}$=5, $n_{wounded}$=12, p=0.532;* ***(c)*** S. curviseta*: $n_{unwounded}$=13, $n_{wounded}$=13, p=0.004.*

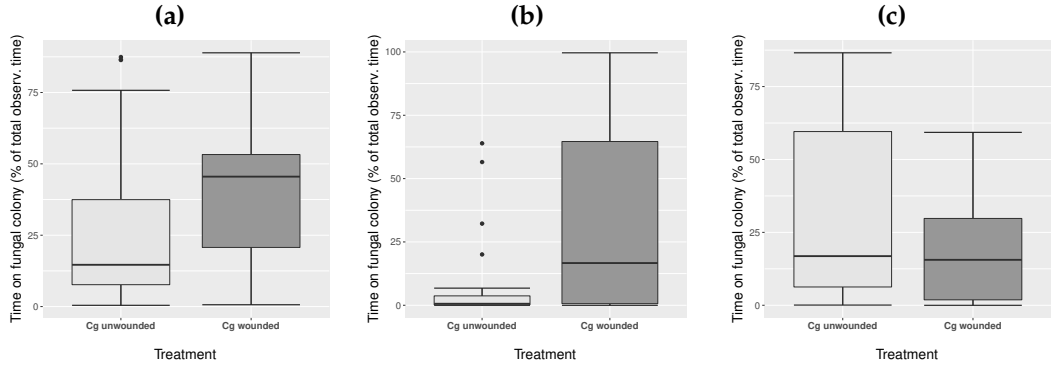

Figure A.2.10: *Contact phase: 'Contact time' (% of total observation time) in response to unwounded and wounded* C. globosum *colonies (n=20).* ***(a)*** F. candida*: p=0.102;* ***(b)*** H. nitidus*: p=0.040;* ***(c)*** S. curviseta*: p=0.280.*

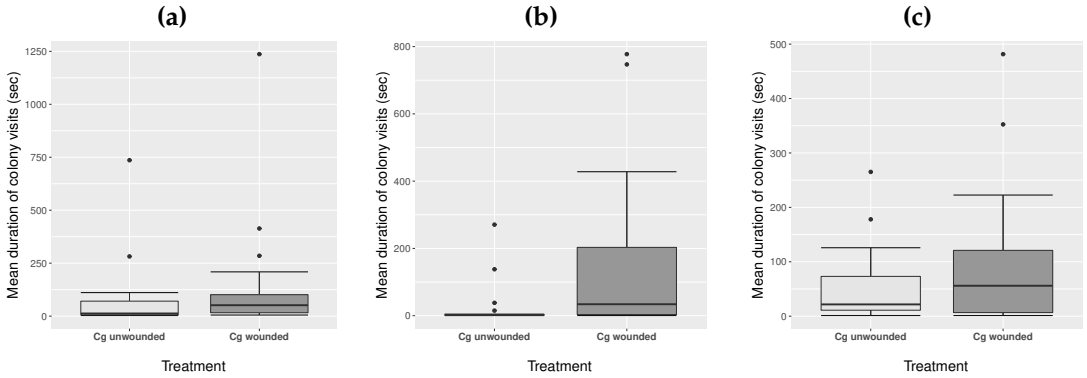

Figure A.2.11: *Contact phase: 'Mean duration of colony contacts' in response to unwounded and wounded* C. globosum *colonies.* ***(a)*** F. candida*: $n_{unwounded}$=20, $n_{wounded}$=20, p=0.043;* ***(b)*** H. nitidus*: $n_{unwounded}$=17, $n_{wounded}$=18, p=0.019;* ***(c)*** S. curviseta*: $n_{unwounded}$=20, $n_{wounded}$=18, p=0.534.*

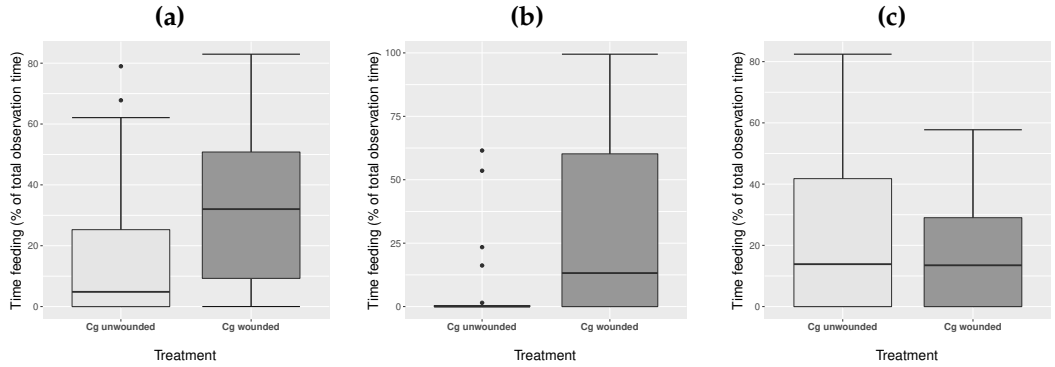

Figure A.2.12: *Contact phase: 'Feeding time' (% of total observation time) in response to unwounded and wounded* C. globosum *colonies (n=20).* ***(a)*** F. candida*: p=0.055;* ***(b)*** H. nitidus*: p=0.013;* ***(c)*** S. curviseta*: p=0.637.*

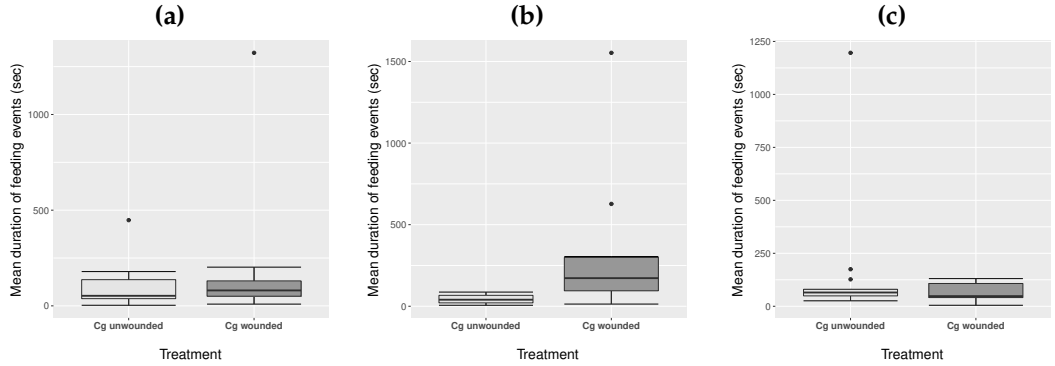

Figure A.2.13: *Contact phase: 'Mean duration of feeding events' in response to unwounded and wounded* C. globosum *colonies.* ***(a)*** F. candida*: $n_{unwounded}$=13, $n_{wounded}$=17, p=0.483;* ***(b)*** H. nitidus*: $n_{unwounded}$=5, $n_{wounded}$=12, p=0.027;* ***(c)*** S. curviseta*: $n_{unwounded}$=13, $n_{wounded}$=13, p=0.479.*

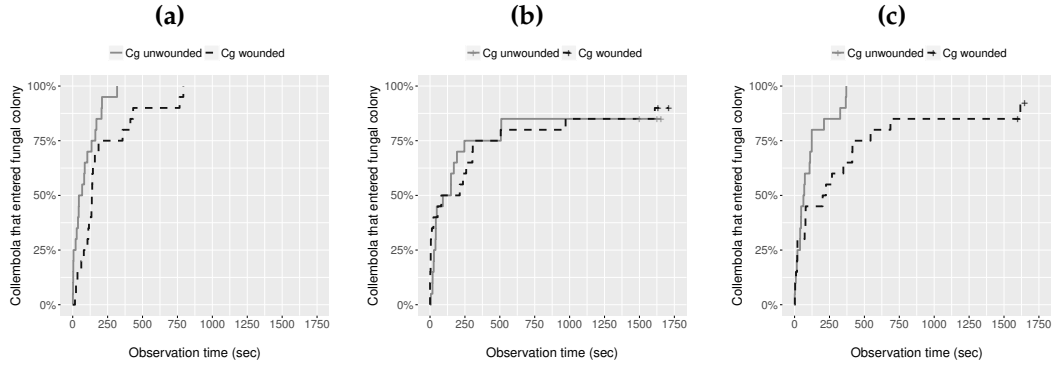

Figure A.2.14: *Contact phase: Kaplan-Meier curves for the 'latency to the first colony contact' in response to unwounded and wounded* C. globosum *colonies (n=20) within an observation period of ~28 minutes.* ***(a)*** F. candida*: p=0.022;* ***(b)*** H. nitidus*: p=0.849;* ***(c)*** S. curviseta*: p=0.014.*

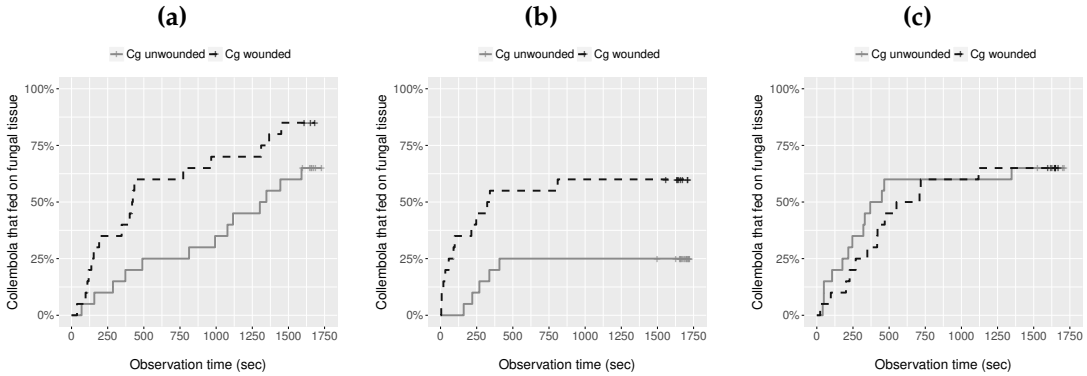

Figure A.2.15: *Contact phase: Kaplan-Meier curves for the 'latency to the first feeding event' in response to unwounded and wounded* C. globosum *colonies (n=20) within an observation period of ~28 minutes.* ***(a)*** F. candida*: p=0.047;* ***(b)*** H. nitidus*: p=0.022;* ***(c)*** S. curviseta*: p=0.692.*

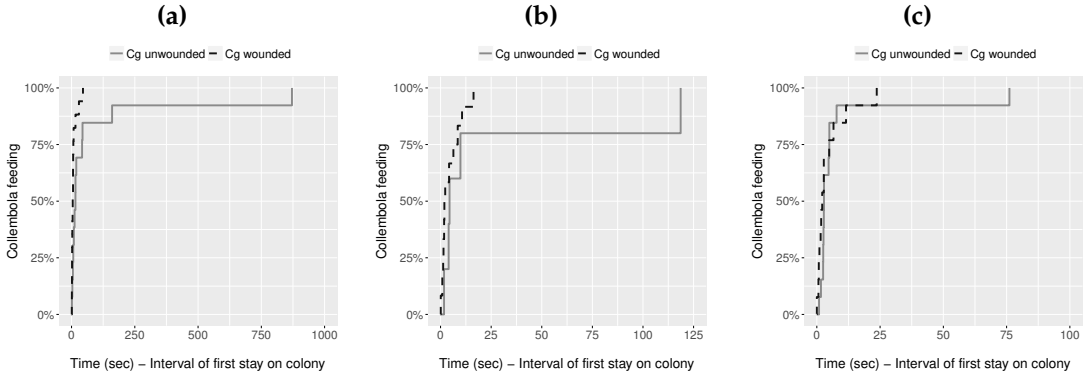

Figure A.2.16: *Contact phase: Kaplan-Meier curves for the 'feeding onset latency after the first colony contact' in response to unwounded and wounded* C. globosum *colonies. Observations without feeding event were excluded.* ***(a)*** F. candida*:* $n_{unwounded}$*=13,* $n_{wounded}$*=17, p=0.021;* ***(b)*** H. nitidus*:* $n_{unwounded}$*=5,* $n_{wounded}$*=12, p=0.263;* ***(c)*** S. curviseta*:* $n_{unwounded}$*=13,* $n_{wounded}$*=13, p=0.452.*

CHAPTER 3

FUNGAL VOLATILES MODULATE THE SPATIAL FORAGING BEHAVIOUR OF THE COMMON WOODLOUSE (*Oniscus asellus*)

3.1 ABSTRACT

As litter-feeders and keystone predators of fungi, isopods significantly contribute to decomposition processes in terrestrial ecosystems. Isopods preferentially feed on microbe-colonised pre-decomposed litter and the presence of microorganisms is assumed to indicate the presence of high-quality, viz. cellulose-rich and easily digestible, food sources. The location and selection of microbe-colonised litter has been suggested to be driven by microbial non-volatile and/or volatile chemical cues. By using an isopod-fungus model system - *Oniscus asellus*, *Chaetomium globosum* - and a direct observational video-tracking approach, this study focuses on the influence of fungal-derived volatiles on the spatial foraging behaviour of isopods. It was hypothesised that isopods use fungal volatiles as infochemicals to locate food from a distance, and that a wound-activated increase in oxylipin volatiles, e.g. 3-octanone, further increases attraction of isopods to their fungal diet. By analysing individual movement patterns, I found that isopods were attracted to and arrested by *C. globosum* volatiles. Wounding of fungal tissue, however, did not change the behaviour of isopods. Using common metabolites emitted by *C. globosum*, 3-octanone and 3-methyl-1-butanol, I show that fungal volatiles may influence isopod foraging patterns by acting as arrestants and/or repellents. However, effects of the single compounds did not explain the observed attraction of isopods to fungal colonies. The results support the hypothesis that isopods use fungal volatiles during foraging and may serve as a conceptual starting point for better understanding the complexity and dynamics of such infochemicals in the searching and feeding decisions of this important group of terrestrial crustaceans in decomposer systems.

Keywords: *crustacean, fungus-arthropod interaction, attraction, arrestance, infochemicals, volatile organic compounds, oxylipins, 3-octanone, 3-methyl-1-butanol*

3.2 INTRODUCTION

Known as generalist detritivores and important decomposers in soil ecosystems, isopods use a variety of different food sources, although plant litter makes up the major part of their diet (Abd El-Wakeil 2015, Carefoot 1993, Hassall and Rushton 1984, Hassall *et al.* 1987, Warburg 1987). While the direct contribution of isopod grazing to decomposition processes via physical and chemical modification of plant material is rather low, a strong indirect contribution via the stimulation of microbial growth has been suggested (Hassall *et al.* 1987).

Isopods preferentially feed on leaf-litter that is colonised by fungi (Gunnarsson 1987, Hassall and Rushton 1984, Soma and Saito 1983, Stöckli 1990, Zidar *et al.* 2003) and the presence of microorganisms - bacteria and fungi - increases the attractivity of plant litter (Ihnen and Zimmer 2008). Moreover, isopods can differentiate between litter-colonising fungi and show selective feeding (Kayang *et al.* 1996, Soma and Saito 1983). Fungi therefore seem to play an important role in the nutrition of terrestrial isopods (Horváthová *et al.* 2016, Soma and Saito 1983, Zimmer and Topp 1998). Gut content analyses indeed revealed that fungal tissue makes up a significant proportion of their diet (Soma and Saito 1983), which contributes to biomass gain of isopods (Zimmer *et al.* 2003). Zimmer *et al.* (2003) assumed that the presence of microorganisms indicates the presence of high-quality and easily digestible food and that specific microbe-derived chemicals may function as phagostimulants for isopods. Recent studies show an immediate contribution of fungal tissue to the diet of isopods and they have thus been classified as keystone predators of fungi, which impact fungal communities on a global scale (Crowther *et al.* 2013).

While the bacteria/fungus-mediated food choice involving direct contact with the food source is well investigated, there is only one study showing that isopods – without prior contact with a substrate – were more strongly attracted to the odour of microbe-colonised litter than to the odour of sterile litter (Zimmer *et al.* 1996). However, as this study does not provide any taxonomic information on the microbial colonisers, it remains unknown whether attraction was mediated by volatiles from bacteria or fungi. For a better understanding of isopods in decomposer community and food web ecology, it is important to reveal the chemical mechanisms underlying the feeding decisions of these keystone foragers. By analysing movement patterns of *Oniscus asellus* woodlice in response to the fungus *C. globosum*, the present study aims to provide a first glimpse into the role of fungal volatiles as infochemicals in the foraging behaviour of isopods.

C. globosum is a common saprotrophic soil fungus that colonises dead plant material and has been shown to be a suitable food source for isopods (Ihnen and Zimmer 2008, Rothe and Gleixner 2000). When grown on a standard microbiology culture medium, the volatile profile of *C. globosum* is dominated by two major compounds, 3-methyl-1-butanol and 3-octanone (Chapter 2, Subsection 2.4.1). 3-methyl-1-butanol is emitted by many bacteria and fungi (Lemfack *et al.* 2018) and has been suggested to be a reliable indicator of microbial activity (Korpi et al. 2009; 1998). 3-octanone is known to be one of the most common volatiles produced by fungi (Börjesson *et al.* 1992, Korpi *et al.* 2009, Schuchardt and Kruse 2009, Schuchardt and Strube 2013). While the abundance of 3-octanone is low in undisturbed *C. globosum* colonies, tissue wounding causes a significant increase of this compound and additionally triggered the formation of 3-octyl acetate (Chapter 2, Subsection 2.4.1). The precursor of 3-octyl acetate, 3-octanol, as well as 3-octanone belong to the so-called oxylipins and derive from the lipoxygenase (LOX)-pathway (Tressl *et al.* 1982). Conserved among plants and fungi, the wound-activated increase of oxylipins represents a common response and is of major importance for cross-kingdom communication and driving interactions between fungi, plants, and animals (Brodhun and Feussner 2011). Several studies on plant-insect interactions have shown that volatile oxylipins, especially six-carbon green leaf volatiles, are crucial in regulating the foraging behaviour of herbivorous insects (e.g. Bruce *et al.* 2005, Schoonhoven *et al.* 2005, Wei and Kang 2011). In an analogous manner, fungal volatile oxylipins possibly act as infochemicals influencing the foraging patterns of fungus-associated arthropods (Holighaus and Rohlfs 2016; 2018). I was therefore especially interested in the response of isopods to the wide-spread fungal ketone 3-octanone. The present study was designed to answer the following questions: (1) Are components of the *O. asellus* foraging behaviour - attraction, repulsion, arrestance - influenced by volatiles emitted by *C. globosum*? (2) If yes, what is the contribution of 3-octanone and 3-methyl-1-butanol? (3) Does the wound-activated release of fungal oxylipins alter isopod responses to *C. globosum*? With this study I aim to provide first insights into how fungal volatiles and dynamics therein contribute to spatial variation in a keystone predator of fungi.

3.3 MATERIAL AND METHODS

3.3.1 FIELD COLLECTION OF *O. asellus* AND CULTIVATION OF *C. globosum*

O. asellus individuals were collected in March 2016 (first experiment with fungal colonies) and September 2017 (second experiment with authentic compounds) from deadwood in the Göttinger Forest, central Germany (51°32′ N, 9°57′ E). Males were identified, transferred to ventilated screw-top jars filled with moist plaster and activated charcoal (40:1), and kept at 20°C in constant darkness. Directly after the field collection the animals were starved for eight days prior to the experiments. The plaster was moisturised regularly. To avoid any sex-specific differences in olfaction and subtle variation in female reproductive stage on the foraging behaviour, only males were used.

C. globosum was cultivated on sterile malt extract agar medium (6 g agar, 9 g malt extract, 1.5 g soy peptone, filled up to 300 ml with H_2O) at 20°C in constant darkness and maintained by transferring pieces of fungal tissue to fresh medium plates regularly. Fungal colonies for the experiments developed for five days on 3 ml malt extract agar in polypropylene cups (25 mm in diameter, 7 mm in height).

TRACKING OF ISOPOD MOVEMENT IN RESPONSE TO FUNGAL COLONIES AND AUTHENTIC COMPOUNDS

The computer software CyberLink PowerDirector® (CyberLink Corporation, Taipei, Taiwan) and an infrared camera were used for video recording of *O. asellus* movement in a no-choice still-air olfactometer at 20°C. Circular glass Petri dishes (19 cm in diameter), filled with 450 ml water agar to provide a moist and smooth surface, were used to define the experimental arena. To obtain indirect and even illumination of the experimental arena, two infrared lamps (Kema Electronic Co., 12-15 V/DC, M120) were installed below within a polystyrene box and covered with a frosted perspex screen (Chapter 2, Figure 2.2). Immediately prior to the recording, a polypropylene cup, containing the respective volatile source, was placed in the centre of the arena (Figure 3.1). The cup was covered with plastic gauze (mesh 2 mm) to prevent isopods from getting into direct contact with the volatile source. Single isopods were released at the edge of the arena and remained trapped in a

glass vial for a two-minute acclimatisation period before starting the 15-minute recording session. After removal of the glass vial, the arena was covered with a glass plate. Observations were carried out on different days with an equal number of replicates per treatment per day (randomised).

In a first experiment isopod movement patterns were observed in response to unwounded and wounded *C. globosum* colonies, and malt extract agar (controls) (n=20). Standardised wounding of fungal tissue was done using a thin wire loop; nearly 50% of the fungal tissue were disrupted by scratching a mesh pattern (four horizontal and four vertical scratches) without injuring the culture medium. In a second experiment, movement patterns of isopods were observed in response to authentic 3-octanone (96%, CAS: 106-68-3, Merck, Darmstadt, Germany), 3-methyl-1-butanol (98.5%, CAS: 123-51-3, Merck), and a solvent control (n=10). By means of gas chromatography-mass spectrometry both compounds were found to be emitted by unwounded as well as wounded *C. globosum* colonies (Chapter 2, Subsection 2.4.1). Whereas headspace concentrations of 3-octanone were significantly increased in wounded colonies, 3-methyl-1-butanol concentrations were not affected by tissue wounding (Chapter 2, Subsection 2.4.1). To mimic 3-octanone concentrations that resemble the ones in the headspace of unwounded and wounded fungi, the compounds were diluted with paraffin (CAS: 8012-95-1, Merck) at concentrations w/w 10^{-6} and 10^{-4} respectively. Pure paraffin served as solvent control treatment. 3-methyl-1-butanol was tested at concentrations of w/w 10^{-6} and 10^{-8}, the latter resembling to quantities measured in the headspace of both wounded and unwounded *C. globosum* colonies (Chapter 2, Subsection 2.4.1). A circular piece of absorbent filter paper (15 mm in diameter) was placed within a polypropylene cup (25 mm in diameter, 7 mm in height) and impregnated with 100 μl compound solution or pure paraffin.

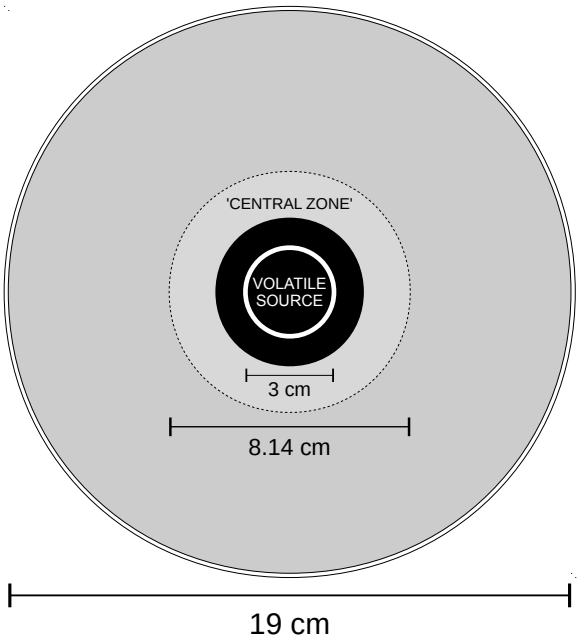

Figure 3.1: *Layout and dimensions of the experimental arena with a 'central zone' defined around the volatile source by means of EthoVision® XT (arena settings) for assessing responses of individual* O. asellus *isopods to* C. globosum *fungal colonies and authentic compounds 3-octanone and 3-methyl-1-butanol. As the sample vessel (polypropylene pod containing the volatile source) caused background noise and consequently interfered with proper detection of the animal, which leads to disruptions of the automated tracking process in EthoVision® XT, the black area enclosing the volatile source was excluded from the experimental arena.*

VIDEO ANALYSIS

Using the semi-automated tracking platform EthoVision® XT (version 8.0; Noldus Information Technology, Wageningen, Netherlands) (Noldus *et al.* 2001) isopod movement patterns were assessed by means of spatial measurements in relation to a 'central zone' that was defined around the volatile source (Figure 3.1). Among a number of variables that were captured and calculated by EthoVision® XT, the following were selected to express attraction and/or arrestance of isopods and to quantify and compare the attractivity of the different volatile sources: (1) 'latency to the first arrival at the central zone', (2) 'duration of the first central zone visit', (3) 'percentage of time in the central zone'. If the natural fungal volatiles or the authentic compounds act as attractants, isopods were expected to enter the 'central zone' more rapidly compared to the fungal-free and the paraffin control treatment respectively. If the volatiles have arresting effects, the animals were expected to reside longer in the 'central zone' compared to the control treatments, immediately after they first visited that zone or relative to the overall amount of time.

3.3.2 STATISTICS

Data was analysed using the R statistical environment (RStudio, version 1.0.153 for Mac OS X, R Development Core Team (2008)). A Cox regression with likelihood estimates was applied on time-dependent event data ('latency to the first arrival at the central zone', 'duration of the first central zone visit') to test whether isopod arrival and departure tendencies differ between treatments. In case of global significance pairwise differences between treatments were tested using Log-Rank tests. Global and per-variable proportionality of hazards was verified by visual examination of Schoenfeld residuals plots and tested by means of goodness-of-fit $\tilde{\chi}^2$ tests (function 'cox.zph'). This diagnostic analysis revealed proportionality with respect to the *C. globosum* fungal treatment, thus a regular unweighted Cox regression was applied (function 'coxph', package 'survival'). Regarding the authentic compounds (full model including 3-methyl-1-butanol and 3-octanone at both concentrations, and paraffin controls) proportionality of hazard rates was violated concerning both time-dependent variables 'latency to the first arrival' (global $\tilde{\chi}^2$=9.85, p=0.043) and 'duration of the first central zone visit' (global $\tilde{\chi}^2$=11.97, p=0.018), i.e. the average hazard ratio for the respective factor is under- or overestimated. Therefore, a weighted Cox regression with different weight per event (function, package 'coxphw', template='AHR') was applied to estimate average hazard ratios as proposed by Schemper *et al.* (2009).

The effect of volatile sources on isopods' 'percentage of time in the central zone' (percentage of total observation time) was tested with a beta regression model (function 'betareg', package 'betareg'). The data was transformed using a method proposed by Smithson and Verkuilen (2006) (formula: $y * (n_{obs-1}) + 0.5)/n_{obs})$) to eliminate zeros and allow for applying a beta regression. For testing the effect of the volatile treatment with respect to fungal colonies (unwounded, wounded) and the medium control, the volatile treatment (explanatory variable) entered the model as categorical variable. An analysis of variance (function 'Anova', package 'car') was applied to test for global significance of the volatile treatment effect. To test for a significant difference between unwounded and wounded colonies Tukey's post-hoc test (function 'glht', package 'multcomp') was used. The effects of the authentic compounds 3-octanone (w/w 10^{-4} and 10^{-6}) and 3-methyl-1-butanol (w/w 10^{-6} and 10^{-8}) on the 'percentage of time in the central zone' were analysed independently and the compound concentration (explanatory variable) entered the model as continuous variable. An analysis of variance (function 'Anova', package 'car') was applied to test for a global significant treatment effect.

3.4 RESULTS

3.4.1 ISOPOD MOVEMENT PATTERNS IN RESPONSE TO UNWOUNDED AND WOUNDED *C. globosum* COLONIES

In the light of the striking wound-activated changes in the volatile profile of *C. globosum*, it was tested whether isopods alter the spatial foraging behaviour in response to unwounded and wounded *C. globosum* colonies. The arrival tendency of isopods at the 'central zones' (variable: 'latency to first arrival') was significantly affected by the presence of *C. globosum* (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=8.32, df=2, p=0.016). Hazard ratios (exp(coef)) indicate that the arrival tendency of isopods in the presence of an unwounded *C. globosum* colony was 2.2 times higher (p=0.024) and in the presence of a wounded colony 2.44 times higher (p=0.010) compared to the tendency to arrive at fungal-free controls (Figure 3.2a). Whereas 16 out of 20 animals arrived in the presence of controls, 19 and 20 animals arrived in the presence of unwounded and wounded *C. globosum* colonies respectively. However, the 'duration of the first central zone visit' was not different between treatments (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=2.83, df=2, p=0.242). A significant effect was also observed with respect to the 'percentage of time in the central zone' (Beta regression, ANOVA Type II, $\tilde{\chi}^2$=13.92, df=2, p<0.001). Compared to the 'percentage of time in the central zone' of fungal-free controls isopods spent a significantly higher percentage of the time in 'central zones' of wounded colonies (Beta regression, z=2.71, df=2, p=0.007) (Figure 3.2b), however, no significant difference was found between the control and unwounded colonies (Beta regression, z=1.88, df=2, p=0.060). A pairwise comparison between unwounded and wounded treatment groups with regard to both 'latency to the first arrival' (Post-hoc test Log-Rank test, df=2, p=0.701) and 'percentage of time in the central zone' (Post-hoc test Tukey, t=-0.85, df=2, p=0.485) revealed that fungal tissue wounding did not affect isopod movement patterns.

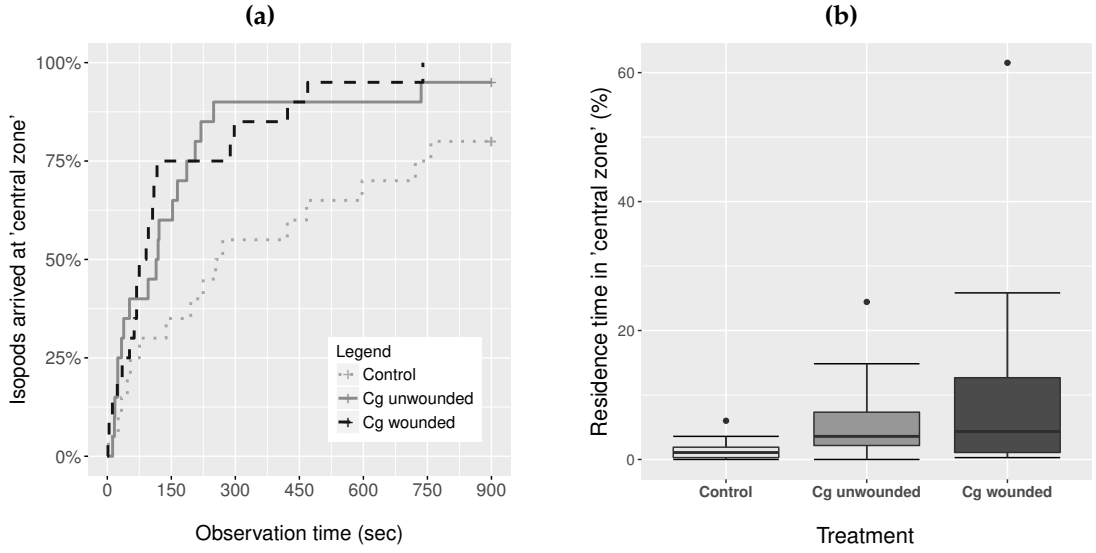

Figure 3.2: *Response of isopods to fungal colonies.* ***(a)*** *Arrival tendencies (variable: 'latency to the first arrival at the central zone') and* ***(b)*** *'percentage of time in central zones' (% of total observation time) of* O. asellus *in the presence of unwounded and wounded* C. globosum *colonies and fungal-free controls (observation period: 15 minutes).*

3.4.2 ISOPOD MOVEMENT PATTERNS IN RESPONSE TO AUTHENTIC COMPOUNDS 3-OCTANONE AND 3-METHYL-1-BUTANOL

O. asellus responses to authentic compounds 3-octanone and 3-methyl-1-butanol were observed to identify those volatile compounds that are causative for previously observed differences in *O. asellus* responses between *C. globosum* colonies and fungal-free controls. A significant global treatment effect was found with respect to the 'latency to the first arrival at the central zone' (arrival tendency) (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=12.18, df=4, p=0.016). Interestingly, comparisons of compound treatments against the solvent control revealed that the tendency of isopods to arrive at the 'central zone' was significantly (63%) lower in the presence of the higher concentration of 3-methyl-1-butanol (Table 3.1) (Figure 3.3a). Arrival tendencies in the presence of 3-octanone and the lower concentration of 3-methyl-1-butanol (10^{-8}), however, did not differ from the control. The 'duration of the first central zone visit' (departure tendency) was not affected by the volatile treatment (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=7.91, df=4, p=0.095). The overall proportion of time spent in the central zone increased with increasing 3-octanone concentration (Beta regression, ANOVA Type II, $\tilde{\chi}^2$=4.24, df=1, p=0.039), yet this time remained unaltered in the

presence of different 3-methyl-1-butanol concentrations (Beta regression, ANOVA Type II, $\tilde{\chi}^2$=0.17, df=1, p=0.681) (Figure 3.3b). The 'duration of the first central zone visit' (departure tendency) was not affected by the volatile treatment (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=7.91, df=4, p=0.095).

Table 3.1: *Cox regression pairwise comparisons of 3-octanone and 3-methyl-1-butanol treatment groups against the control group with respect to the 'latency to the first arrival at the central zone' (arrival tendency). Effect size is represented by the hazard ratio ($\exp(coef)$).*

Comparison	N	*Events*	Z	$\exp(coef)^{(*)}$	$P-value$
Control - 3-octanone 10^{-4}	20	19	0.29	1.17	0.768
Control - 3-octanone 10^{-6}	20	19	-0.90	0.67	0.367
Control - 3-methyl-1-butanol 10^{-6}	20	18	-2.52	0.37	**0.012**
Control - 3-methyl-1-butanol 10^{-8}	20	17	0.27	1.18	0.784

$^{(*)}$ Given hazard ratios relate to the respective authentic compound treatment group. A value lower than 1 indicates a decreased tendency of isopods to arrive at the 'central zone'.

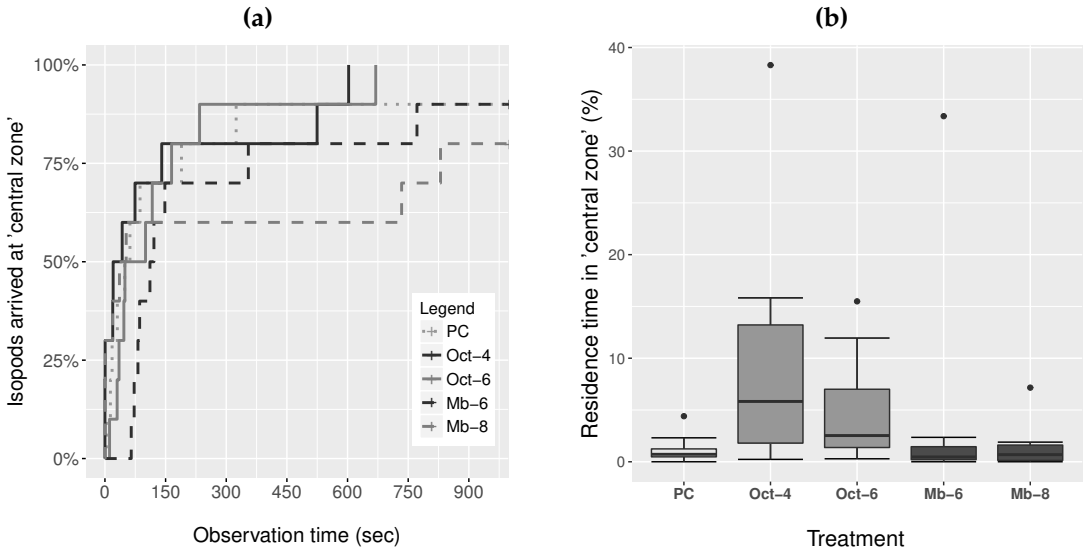

Figure 3.3: O. asellus ***(a)*** *arrival tendencies (variable: 'latency to the first arrival at the central zone') and* ***(b)*** *'percentage of time in the central zone' (% of total observation time) in response to 3-octanone in concentrations of w/w 10^{-6} (Oct-6) and 10^{-4} (Oct-4), 3-methyl-1-butanol in concentrations of w/w 10^{-8} (Mb-8) and 10^{-6} (Mb-6), and paraffin controls (PC) within an observation period of 15 minutes.*

3.5 DISCUSSION

Previous studies have suggested that food location by *O. asellus* isopods requires physical contact with the food source (Gunnarsson 1987, Zidar *et al.* 2003). The results of the present study provide first evidence of a role of fungal-borne volatiles in establishing contact between *O. asellus* and a dietary saprotrophic fungus. A higher 'arrival tendency' and 'percentage of time in the central zone' in the presence of *C. globosum* colonies indicate that isopods are attracted, and in addition, arrested in close proximity to fungal colonies. This supports the hypothesis that isopods use fungal volatiles as information to locate food from a distance and agrees with Zimmer *et al.* (1996), who reported that *Porcellio scaber* isopods are attracted to volatiles deriving from litter-colonising microorganisms rather than volatiles of the litter itself. Thus, the use of microbial/fungal volatiles is supposed to be a mechanism in terrestrial isopod foraging to locate favourable microhabitats characterised by high microbial activity.

Since wounding of fungal tissue activates volatile oxylipin emissions, it was hypothesised that isopods locate microhabitats with high microbial and detritivore activity more easily and, therefore, are more strongly attracted to wounded *C. globosum* colonies than to unwounded colonies. Unexpectedly, isopods were attracted to and arrested by unwounded and wounded *C. globosum* colonies to the same extent. In line with this, responses of isopods to the authentic compound 3-octanone indicate that this oxylipin compound did not elicit attraction. The higher percentage of time spent in close proximity to the 3-octanone source, however, indicates that isopods were arrested by this compound. The second *C. globosum* authentic compound that was tested for attracting isopods, 3-methyl-1-butanol, is known to derive from dead plant material that is colonised by microorganisms (Bjurman 1997, Isidorov and Jdanova 2002, Wheatley *et al.* 1996) and was repeatedly found to mediate attraction in fungus-arthropod interactions (Becher *et al.* 2012, Lin and Phelan 1991, Phelan and Lin 1991, Rizvi and Raman 2016, Stötefeld *et al.* 2015, Tasin *et al.* 2012). However, the results show that this compound did not attract *O. asellus* isopods; on the contrary, a concentration of w/w 10^{-6} of 3-methyl-1-butanol apparently had a deterrent effect. The results indicate that both volatile compounds, 3-octanone and 3-methyl-1-butanol, affect the foraging behaviour of isopods, but isopod responses to individual compounds do not explain the observed attraction of isopods to *C. globosum* colonies. The fact that isopods were attracted to *C. globosum* but not to the main compounds produced by this dietary fungus is surprising. Two possible explanations for these contradictory results are conceivable: (1) synergistic interactions

of volatile compounds elicited attraction to *C. globosum*; (2) other *C. globosum*-derived compounds, not tested in the present study, attracted isopods. Possible candidate volatiles that come into question to be responsible for isopod attraction to *C. globosum* colonies are highly volatile compounds, e.g. carbon dioxide or short-chain alcohols. GC-MS analysis revealed the presence of carbon dioxide and ethanol in headspace samples of both fungal-free malt extract agar controls and *C. globosum* colonies (Chapter 2, Subsection 2.4.1). Quantities of respective compounds and differences between control and fungal samples, however, could not be determined due to overlap and interference of the compound peaks. Therefore, one can only speculate that these compounds were responsible for or at least contributed to isopod attraction. In the light of the findings that the number of isopods captured in the field is positively correlated with cellulytic and respiratory activity of microorganisms (Zimmer and Topp 1999) and that fungi facilitate the exploitation of cellulose rich plant detritus and thus benefit individual isopod fitness (Uesbeck and Topp 1995, Zimmer and Topp 1997), it is likely that isopods are attracted to volatiles that relate to microbial respiration (Öhlinger *et al.* 1996), indicating microbial activity and the presence of suitable food sources.

The significance of fungal volatiles in mediating fungus-arthropod interactions similar to that of isopods and fungi, viz. interactions in which fungi act as niche constructors by paving the way for invertebrate colonisers via modification of plant material, was demonstrated with regard to Coleoptera (Belmain *et al.* 2002, Blackmer and Phelan 1991, Hulcr *et al.* 2011, Lin and Phelan 1991, Nout and Bartelt 1998, Phelan and Lin 1991), Diptera (Becher *et al.* 2012, Buser *et al.* 2014, Dobzhansky *et al.* 1956, Fischer *et al.* 2017, Palanca *et al.* 2013, Stötefeld *et al.* 2015), and Lepidoptera (Mondy *et al.* 1998, Rizvi and Raman 2016, Tasin *et al.* 2011; 2012, Witzgall *et al.* 2012). Volatile compounds that were repeatedly found to mediate attraction in respective systems are mainly alcohols, aldehydes, and acetates (Becher *et al.* 2012, Lin and Phelan 1991, Nout and Bartelt 1998, Phelan and Lin 1991, Rizvi and Raman 2016, Stötefeld *et al.* 2015, Tasin *et al.* 2012) - common compounds that are known to be produced by a wide range of fungi and other microorganisms. These studies suggest that arthropods associated with microbe-infected plant material use common ubiquitous fungal/microbial volatiles to detect and locate preferred feeding and/or oviposition sites. The present study provides first evidence that isopods also use fungal volatile cues during foraging, but further work is needed to identify those volatiles and possible synergistic effects that mediate the here observed attraction response.

In conclusion this study clearly shows that fungal volatiles influence the spatial foraging behaviour of isopods and are used as infochemicals to locate food from a distance. Whereas ar-

restance of isopods in the presence of *C. globosum* can be attributed to the authentic oxylipin volatile 3-octanone, attraction to fungal colonies could not be explained by the single compound effects. Contrary to expectations, wounding of fungal tissue and related activation/increase in oxylipin emissions did not further increase the attractivity of fungal colonies. Further research is needed to explain the here observed behavioural responses and to fully understand the role of fungal volatiles in mediating isopod-fungus interactions. Future experiments need to aim at the identification of those fungal authentic compounds responsible for isopod attraction, particularly by investigating isopod responses to further common fungal volatiles and with regard to possible synergistic effects.

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CHAPTER 4

VOLATILE-MEDIATED FORAGING RESPONSES TO YEASTS AND FILAMENTOUS FUNGI CORRELATE WITH FUNGIVORE GROWTH AND REPRODUCTION

4.1 ABSTRACT

Existing research provides evidence for a significant role of fungal volatiles as cues in affecting the foraging behaviour of soil-living fungivorous arthropods by acting as attractants, deterrents, and/or arrestants. Here I tested the hypothesis that hemiedaphic Collembola (*F. candida*) respond differently to differences in fungal volatile emissions to discriminate between fungi of varying suitability by analysing volatile profiles of different yeast (*Cryptococcus terricola*, *Trichosporon dulcitum*, *Metschnikowia pulcherrima*) and filamentous fungi (*Aspergillus nidulans*, *Penicillium expansum*, *Trichoderma harzianum*) (GC-MS), observing behavioural responses of single Collembola during both the searching and the contact phase of food selection by means of the computer software packages EthoVision® XT and Observer® XT (Noldus), and evaluating fitness consequences (growth, reproduction) of the respective fungal diets.

Volatile-mediated responses and most notably feeding decisions (acceptance or rejection of fungal food sources) were largely reflected in Collembola fitness measures. In detail, the volatile bouquets of the yeasts, which were composed of only a small number of alcohols and acetates, mediated attraction or arrestance, were accepted as food source and positively affected Collembola growth and reproduction (number of eggs). In case of the filamentous fungi *A. nidulans* and *T. harzianum* behavioural responses were hardly reflected in Collembola fitness measures. The volatile bouquets of both fungi did not elicit any response. *T. harzianum* was initially rejected as food source, however, when given as single diet over several days, Collembola increased growth and laid eggs. In contrast, the filamentous fungus *P. expansum* was rejected when direct contact was possible and the results of the fitness assay indicate that this fungus is unsuitable as food source for *F. candida*. In line

with this, the volatile bouquet of *P. expansum*, which was found to be characterised by the presence of several terpenoid compounds, deterred the animals. One particular compound, namely geosmin, which is known to elicit avoidance in adult fruit flies, was tested on affecting Collembola movement patterns and found to be responsible for the deterrent effect, at least to some extent. In agreement with the assumption that fungi have evolved chemical defence strategies against antagonists, I suggest that the emission of deterrent volatiles represents an effective chemical defence mechanism against fungivory.

I conclude that the use of fungal volatiles as cues is important for Collembola to increase their fitness. *F. candida* exhibits food-specific foraging strategies, strongly depending on fungal chemical properties and presumably also on fungal growth characteristics. Unicellular yeasts may be better accessible as food source than filamentous fungi. Whether morphological properties of the here tested fungi play a role in affecting their acceptance as food source remains to be tested.

Keywords: *Collembola, fungus-arthropod interaction, foraging behaviour, food selection, fitness, yeasts, filamentous fungi, volatile organic compounds, wounding, oxylipins, geosmin*

4.2 INTRODUCTION

As main decomposers and colonizers of litter, fungi make up the major part of the total soil microbial biomass (Kjoller and Struwe 1982, Schnürer *et al.* 1985) and constitute an abundant food source for soil invertebrate decomposers and fungal grazers, including isopods (Crowther *et al.* 2013), beetles (Harrington 2005), millipedes, springtails, acarids, nematodes, annelids, and gastropods (Maraun *et al.* 2003) (Chapter 1). Terrestrial soil ecosystems harbour more than 3300 species of filamentous fungi (Gams 2007) and up to 130 yeast species (Esteve-Zarzoso *et al.* 1999). Differences in chemical properties between fungi, viz. the presence of toxic and/or deterrent secondary metabolites, influence foraging and food selection by fungivorous arthropods and may contribute to the occurrence of selective fungal grazing and preferences for certain fungi (Böllmann *et al.* 2010, Döll *et al.* 2013, Rohlfs and Churchill 2011, Stötefeld *et al.* 2012). There is growing evidence that fungal-derived volatile compounds influence the foraging behaviour of fungivorous soil-living arthropods by acting as attractants, arrestants, repellents, and/or stimulants (Davis *et al.* 2013, Holighaus and

Rohlfs 2018). Using a Collembola-fungus model system I aim to investigate the role of fungal volatiles in affecting foraging decisions and success of fungivorous soil arthropods in further detail by analysing fungal volatile profiles, behavioural responses of Collembola during both the searching and the contact phase of food selection (Schoonhoven *et al.* 2005), and consequences of different fungal diets for Collembola growth and reproduction.

Collembola, a major group of secondary decomposers, are often considered as generalist feeders (Hopkin 1997, Scheu and Setälä 2002). However, several studies attribute a significant role to fungi in the nutrition/diet of Collembola (e.g. Hopkin 1997, Moore *et al.* 1987, Ponge 1991, Rusek 1998). The existence of different dietary preferences among Collembola species for different fungi even indicates a certain degree of specialisation (Bardgett *et al.* 1993, Heděnec *et al.* 2013, Klironomos *et al.* 2008, Larsen *et al.* 2008, Men'ko *et al.* 2006). In many instances grazing preferences were found to be reflected in Collembola fitness measures (e.g. Jørgensen *et al.* 2008, Klironomos *et al.* 2008, Men'ko *et al.* 2006, Rotheray *et al.* 2009); however, there is only one study that related fitness consequences of different fungal diets to volatile-mediated foraging decisions. Sadaka-Laulan *et al.* (1998) have shown that Collembola (*Onichiurus sinensis*) preferences for/avoidance of odour solutions of certain filamentous fungi largely correlate with their feeding decisions and fitness (growth, survival, resproduction) and provide first indication that Collembola are able to detect less suitable fungi by means of volatiles. The relevance of fungal volatiles in affecting Collembola foraging has also been demonstrated by Bengtsson *et al.* (1988; 1991; 1994), Hedlund *et al.* (1995), and Staaden *et al.* (2011). To test the hypothesis that Collembola are able to use fungal-derived volatiles as cues to differentiate between fungal food sources of varying suitability from a distance, I use two different groups of fungi, yeasts and filamentous fungi.

Although yeasts have frequently been shown to be engaged in mutualistic interactions with invertebrates (Blackwell 2017), to be a suitable food source for different fungivorous invertebrates - Collembola (Men'ko *et al.* 2006), Diptera (Anagnostou *et al.* 2010), Lepidoptera (Witzgall *et al.* 2012), Coleoptera (Davis 2015), Annelida (Yurkov *et al.* 2008), Diplopoda (Byzov *et al.* 1993), and Isopoda (Zimmer 2002) - and to be essential for the acceptance of leaf litter as food source by Collembola (Men'ko *et al.* 2006), the present study for the first time investigates the role of yeast-derived volatiles in modulating the foraging behaviour of Collembola. Filamentous fungi and unicellular growing yeasts differ in their chemical properties that may determine their suitability as food source. The production of detrimental volatile and non-volatile secondary metabolites, which is characteristic for a range of filamentous fungi, including those species tested in the present study, reduces their suitability as food

source and is assumed to function as chemical defence against fungivory (Caballero Ortiz *et al.* 2013, Döll *et al.* 2013, Holighaus and Rohlfs 2018, Rohlfs 2015, Rohlfs *et al.* 2007, Spiteller 2008, Stötefeld *et al.* 2012). In contrast to yeast volatile profiles that often comprise alcohols, acids, esters, and ketones, many species of filamentous fungi additionally produce a range of terpenes and compose volatile profiles that are more characteristic and diverse. Many terpenes are known to function as infochemicals (Gershenzon and Dudareva 2007); for example the terpenoid geosmin, which is produced by the here tested fungus *P. expansum*, has been shown to reduce the attractiveness of vinegar to fruit flies (Becher *et al.* 2010) and is suspected to be of great ecological relevance for the detection of harmful microbes by fruit flies (Stensmyr *et al.* 2012). In the light of the differences between yeasts and filamentous fungi I assume that the former present a more suitable food source. I hypothesise that the volatile bouquet of yeasts is more attractive and that yeasts are more accepted as food than filamentous fungi.

An additional focus of this study is on investigating the potential of fungal tissue wounding and related changes in fungal volatile profiles in affecting Collembola foraging decisions. While mechanical wounding/disruption of fungal tissue was shown to activate the emission of oxylipin volatiles in filamentous fungi (Brodhun and Feussner 2011, Combet *et al.* 2009, Fäldt *et al.* 1999, Wurzenberger and Grosch 1983) (Chapter 2, Subsection 2.4.1), a similar systemic response is unlikely for yeasts due to their unicellular structure and has never been described. As fungi are constantly exposed to grazing, the release of wound-activated oxylipin volatiles may have crucial significance in affecting the behaviour of fungivorous arthropods and fitness of both fungi and arthropods. In fact, some oxylipin volatiles were demonstrated to function as cues for different soil invertebrates from a distance (Holighaus and Rohlfs 2018), however, the results from Chapter 2 and Chapter 3 indicate that neither Collembola nor isopods change their searching behaviour (movement patterns) in response to increased headspace concentrations of oxylipins (3-octanone, 3-octyl acetate) in wounded colonies of the saprotrophic fungus *C. globosum* (Chapter 2, Chapter 3). However, wounded *C. globosum* colonies were more accepted as food source by *F. candida*, *H. nitidus*, and *S. curviseta* when direct contact was possible (Chapter 2, Subsection 2.4.3). Moreover, the oxylipin 3-octanone triggered test-biting behaviour in *F. candida* and *S. curviseta* (Chapter 2, Subsection 2.4.4). By including further species of filamentous fungi, the aim is to test in more detail whether Collembola searching patterns in principle remain unaffected by wounding-related changes in the fungal chemistry and whether an increase in oxylipin headspace concentrations in general increases the acceptance of wounded fungal colonies as food source.

4.3 MATERIAL AND METHODS

4.3.1 FUNGAL INCUBATION

Fungal colonies used in the experiments were reared from conidia suspensions in case of filamentous fungi *A. nidulans*, *T. harzianum*, and *P. expansum* and cell suspensions in case of *C. terricola*, *M. pulcherrima*, and *T. dulcitum* yeasts. Due to differences in growth characteristics, yeasts and filamentous fungi were handled differently to obtain suspensions for the experiments.

Yeast cell cultures were initially raised until the saturation point in malt extract broth fluid culture (15 g malt extract; 2.5 g peptone; filled up with water to 500 ml) and stored in a refrigerator at 6°C in darkness. For the preparation of yeast cell cultures for the experiments, inactive saturated suspensions were reactivated by diluting them to a concentration of 10^{-1} with malt extract broth, thereupon fresh suspensions were incubated for 3 days at room temperature on a laboratory shaker (500 rpm), and afterwards, again diluted to a concentration of 10^{-1} with malt extract broth. Obtained suspensions were used for a maximum of 7 days for inoculation of yeast colonies for the experiments, and in the meantime, stored at 6°C in darkness.

Fungal colonies of *A. nidulans*, *T. harzianum*, and *P. expansum* were maintained by regular fresh inoculation and incubation on sterile malt extract agar (MEA) medium. Spore suspensions of these species were obtained by rinsing off conidia from established colonies with sterile Ringer solution. These raw suspensions were then stored at 6°C. At the onset of an experiment, raw suspensions were diluted to the desired concentrations with Ringer solution.

For experiments sterile malt extract agar was inoculated with the respective cell or spore suspension and incubated at 20°C (climate chamber; Lovibond®) in constant darkness. According to growth properties incubation times differed between fungal species (Table 4.1).

Genotypes A and B of *P. expansum* were provided by the Technical University of Denmark (Department of Biotechnology and Biomedicine) and correspond to identification numbers 30506 and 23705, respectively, as listed in the IBT culture collection.

Table 4.1: *Fungal species and genotypes used in experiments.*

Phylum	*Species*	*Genotype*	*Growth stage*	*Incubation time*	*Experiments*(*)
Ascomycota	*Aspergillus nidulans*	veA+	vegetative	8 days	a
			sporulating	10 days	a
		veA1	vegetative	8 days	a
			sporulating	10 days	a,b,c,d
Ascomycota	*Trichoderma harzianum*	T12	vegetative	4 days	a
			sporulating	8 days	a,b,c,d
Ascomycota	*Penicillium expansum*	A	vegetative	2 days	a
			sporulating	4 days	a,b,c,d
		B	vegetative	2 days	a
			sporulating	4 days	a
Ascomycota	*Metschnikowia pulcherrima*			4 days	a,b,c,d
Basidiomycota	*Cryptococcus terricola*			4 days	a,b,c,d
Basidiomycota	*Trichosporon dulcitum*			4 days	a,b,c,d

(*) This column displays the experimental setups in which the respective species, genotype, and growth stage was tested.
a = GCMS volatile profiling, b = Collembola movement patterns (searching phase), c = Collembola behaviour during the contact phase, d = Collembola fitness assay

4.3.2 CULTURING OF *F. candida* COLLEMBOLA

F. candida Collembola cultures ('Berlin' strain) were maintained in the laboratory at 20 °C in constant darkness in Petri dishes (9 cm in diameter) on a mixture of plaster and activated charcoal (proportion 40:1). Aeration was ensured by providing the lids of the Petri dishes with a ventilation opening that was covered with fine mesh gauze. Once per week the Collembola were fed with dried baker's yeast and regular moistening prevented the animals from drying out. Adult individuals were transferred to new Petri dishes for oviposition to raise age-synchronized populations. The age of the Collembola used for behavioural observations ranged between 60 and 90 days. Young adults (25 days old), ready for oviposition and with potential for further increase in body size, were employed for the fitness experiment. Seven days before start of an experiment animals were deprived of food.

4.3.3 SPME-GC-MS VOLATILE PROFILING

By means of the solid phase microextraction (SPME) technique (Pawliszyn 1997) in combination with gas chromatography-mass spectrometry (GC-MS) volatiles were sampled and identified from vegetative and sporulating colonies of filamentous fungi *A. nidulans* (two genotypes), *T. harzianum*, *P. expansum* (two genotypes), and *C. terricola*, *M. pulcherrima*, and *T. dulcitum* yeast colonies (Table 4.1) to reveal differences between and characteristics of volatile profiles from the two fungal life forms. Different genotypes and growth stages of filamentous fungi were analysed and, based on the highest diversity of volatile compounds, I chose one genotype and one growth stage per fungal species for behavioural observations and the fitness assay. In addition, the effect of tissue wounding on volatile emissions was investigated with respect to *A. nidulans*, *T. harzianum*, and *P. expansum* (including all genotypes and growth stages) (Table 4.1). Considering all species, genotypes, growth stages and the wounding treatment, there were 23 fungal treatments, each replicated six times. Evenly distributed over the GC-MS analysis period, volatile profiles of ten fungal-free MEA controls and one empty glass tube were measured.

Fungi were inoculated and incubated for different time periods (Table 4.1) in sterile glass tubes (150 mm in height, 30 mm in diameter) on 50 ml MEA medium. To achieve optimal growth, *A. nidulans*, *T. harzianum*, and *P. expansum* were inoculated with 10 μl conidia suspension (1000 conidia/μl) whereas *C. terricola*, *M. pulcherrima*, and *T. dulcitum* were inoculated with 50 μl cell suspension (undefined cell concentration) as it turned out that these yeasts grow best in a moister environment. During incubation the glass tube samples were sealed with sterile aluminium foil to avoid contamination and ensure air exchange, and stored in separate boxes to prevent potential cross-influence by volatile leakage. Standardised wounding/disruption of fungal tissue was done by scratching a mesh pattern (four horizontal and four vertical scratches) with a thin wire loop without disrupting the culture medium. Approximately 50% of the fungal tissue were disrupted. The wounding treatment was done exactly 30 minutes before volatile sampling and immediately afterwards the glass tubes were sealed with parafilm to allow volatiles to accumulate. Untreated colonies and MEA controls were handled identically except from the wounding treatment. For sampling of volatiles from the fungal headspace, the SPME-fibre (StableFlexTM), with a thickness of 85 μm and CarboxenTM/Polydimethylsiloxane (PDMS) coating, was carefully stuck through the parafilm and exposed to the headspace for exactly 45 minutes. In preliminary GC-MS test runs a sampling period of 45 minutes turned out to be optimal to capture detectable

amounts of low volatile compounds on the one hand, and allow clear differentiation between chromatogram peaks of high volatile compounds on the other.

Fungal volatile profiles were successively analysed by using a GC (Agilent Technologies, 6890N; Palo Alto, USA; non-polar HP-5ms column, 0.25 mm ID, 30 m length, 0.25 μm film thickness) which was coupled to an MS (Agilent Technologies, 5973N; Palo Alto, USA; 20-345 amu, electron ionization at 70 eV). To cover a broad range of high and low volatile compounds and to achieve clear separation between compound peaks the initial GC temperature was set at -30 °C (hold for 1.5 min), followed by a temperature ramp with 6 °C per minute up to 200 °C, and maintenance of 200 °C for 3 minutes. Retention indices were obtained from retention times of target compounds and n-alkanes (C3-C18) by means of the Van den Dool and Kratz equation ($I_x = 100n + 100(t_x - t_n)/(t_{n+1} - t_n)$; t_x: retention time of target compound; t_n, t_{n+1}: retention times of two consecutive n-alkanes eluted immediately before and after t_x). MSD ChemStation Data Analysis software (version D.02.00.275; Agilent Technologies), AMDIS (version 2.66; Geithersburg, MD), and Mass Finder 4 (Hochmuth et al., www.massfinder.com) were used to determine peak identities. Retention indices and mass spectra of target compounds were compared to literature retention values listed in the NIST Chemistry WebBook (NIST Standard Reference Database Number 69, http://webbook.nist.gov/chemistry/) and entries in mass spectral libraries NIST 08, Wiley 9, and Mass Finder 4 Terpenoids Library. In addition, chromatogram raw data was checked with the online software SpectConnect (Styczynski *et al.* 2007) to ensure that none of the compound peaks had been overlooked. For further verification, retention indices of target compounds were compared to those of authentic standards (if available) (Subsection 4.3.4), which were measured with identical GC parameters.

Headspace concentrations of the *P. expansum* characteristic compound geosmin were determined by means of an authentic standard calibration curve comprising four concentrations (10^{-3}-10^{-6}) (Figure A.4.1). 10 mg of geosmin were solved in 100 μl methanol. To obtain a concentration of 10^{-3}, 10 μl of the geosmin-methanol solution were pipetted on the surface of 1000 μl paraffin. After complete evaporation of the methanol (subsequently verified by GC-MS analyses), the remaining geosmin was mixed in the paraffin with a vortex mixer to get a homogeneous solution. This initial solution was used to prepare the concentration series for the quantification of geosmin concentrations by GC-MS. The same geosmin solutions were subsequently used in the off-patch searching experiment (Section 4.3.5).

4.3.4 AUTHENTIC STANDARDS FROM COMMERCIAL SUPPLIERS

Following authentic standards were obtained from commercial suppliers and used for retention index-based identification of fungal derived volatile compounds. Furan (CAS: 110-00-9), hexane (CAS: 110-54-3), 2-methyl-3-buten-2-ol (CAS: 115-18-4), 2-methyl-1-propanol (CAS: 78-83-1), 3-methyl-3-buten-2-ol (CAS: 763-32-6), 3-methyl-1-butanol (CAS: 123-51-3), 3-octanone (CAS: 106-68-3), 3-octanol acetate (CAS: 4864-61-3), 2-methylisoborneol (CAS: 2371-42-8), geosmin (CAS: 19700-21-1).

4.3.5 EXPERIMENTAL SETUPS

SEARCHING PHASE: TRACKING OF COLLEMBOLA MOVEMENT IN RESPONSE TO FUNGAL COLONIES

To test the hypothesis that Collembola are more strongly attracted to and/or arrested by the volatile bouquet of yeast fungi than by filamentous fungi, I investigated the volatile-mediated movement of single Collembola in response to *C. terricola*, *M. pulcherrima*, and *T. dulcitum* colonies, and sporulating colonies of *A. nidulans*, *T. harzianum*, and *P. expansum* by video observation. It is further hypothesised that wound-activated changes in volatile profiles of *A. nidulans*, *T. harzianum*, and *P. expansum* (Subsection 4.4.1, Table 4.3) do not affect Collembola movement patterns.

Fungal colonies were incubated in sterile polypropylene cups (7 mm in hight, 25 mm in diameter) on 3 ml MEA for different time periods depending on fungal growth properties (Table 4.1). In case of *A. nidulans* and *P. expansum* inoculation volume was 10 μl (1000 spores/μl), and with respect to *T. harzianum* which grows optimal in drier environments, 5 μl spore suspension (2000 spores/μl) were used. In case of yeasts 50 μl cell suspension were used for inoculation. Fungal-free MEA served as control. Wounding of fungal tissue was done as described above (Subsection 4.3.3). The experimental arena, a glass Petri dish with a diameter of 10.1 cm (Figure 4.1), was filled with 45 ml water agar to create a surface which allows for unhindered movement and provides sufficient humidity. A fungal colony or fungal-free control was placed centrally in the arena immediately before start of the 15-minute recording. The arena was covered with a glass plate and a single Collembola

was released at the outer edge of the arena and remained trapped in a glass tube for 2 minutes to acclimate. CyberLink PowerDirector® (CyberLink Corporation, Taipei, Taiwan) was used for recording. Recordings were carried out on several days with an equal number of observations per treatment per day (30 replicates, in total 300 recordings).

SEARCHING PHASE: TRACKING OF COLLEMBOLA MOVEMENT IN RESPONSE TO GEOSMIN

As Collembola arrival tendencies were significantly lower in the presence of both unwounded and wounded *P. expansum* colonies compared to medium controls (Section 4.4.2) it is hypothesised that the filamentous fungus *P. expansum* produces at least one volatile compound that has a deterrent effect on foraging *F. candida* individuals. GC-MS analysis revealed that, besides some sesquiterpenes, *P. expansum* emits four terpenoid compounds, namely geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), 2-methylisoborneol, and it's dehydration products 2-methyl-2-bornene and 2-methylenebornane (Martin *et al.* 1988a), that distinguish the volatile profile of *P. expansum* from that of other fungi considered in this study. The compounds geosmin and 2-methylisoborneol are produced by various fungi, streptomycetes, and algae, and are known for an earthy/musty smell (Mahmoud and Buettner 2016, Martin *et al.* 1988b). Geosmin was tested for affecting *F. candida* movement patterns and being responsible for the observed deterrence of Collembola by *P. expansum* colonies using the same experimental setup as described above (Section 4.3.5).

Responses of Collembola were observed in the presence of geosmin in a concentration of 10^{-3}. The average headspace concentration of geosmin in chromatograms of unwounded and wounded *P. expansum* colonies is 25^{-4} Table A.4.1. In addition, a second much lower concentration (10^{-5}) of geosmin was tested to find out how sensitive Collembola respond and if small amounts of this compound suffice to cause deterrence. As geosmin was initially solved in methanol and, after evaporation of methanol, further diluted with paraffin (for detailed preparation of dilutions see Subsection 4.3.3), the geosmin-paraffin dilutions used in this experiment were previously checked for methanol residues to prevent potential solvent-caused bias of Collembola responses. Before video recording, polypropylene cups (25 mm in diameter, 7 mm in height) were provided with circular pieces of absorbent filter paper (13 mm in diameter). The filter paper was soaked with 40 μl of the respective solution.

CONTACT PHASE: OBSERVATION OF COLLEMBOLA ON-PATCH BEHAVIOUR IN RESPONSE TO FUNGAL COLONIES

It is hypothesised that yeast fungi present a more suitable food resource for fungivorous arthropods than filamentous fungi and that higher suitability is reflected in higher acceptance of yeast as food by Collembola. To test this I investigated the on-patch behaviour (direct contact) of individual Collembola in response to *C. terricola*, *M. pulcherrima*, and *T. dulcitum* yeast colonies and sporulating colonies of filamentous fungi *A. nidulans*, *T. harzianum*, and *P. expansum* by means of video observation. Furthermore, it is hypothesised that fungal tissue wounding increases the acceptance of wounded colonies of *A. nidulans*, *T. harzianum*, and *P. expansum* as food source.

Fungal colonies were grown on circular pieces of absorbent filter paper (13 mm in diameter) (Macherey-Nagel, Germany; Type MN 85/70 BF, Ø 90 mm), which were previously dipped in sterile MEA and additionally coated with 80 μl MEA by pipetting. The medium was inoculated with 10 μl spore (1000 conidia/μl) or cell suspension with respect to filamentous fungi *A. nidulans*, and *P. expansum*, and yeasts. For optimal growth of *T. harzianum* colonies, 5 μl spore suspension (2000 conidia/μl) were used for inoculation. Colonies were incubated in sterile Petri dishes (9 cm in diameter) which were stored in ventilated boxes. The boxes were provided with moist paper towels to prevent desiccation of fungal colonies. Fungal-free MEA controls were treated similarly. Incubation times depended on fungal growth properties (Table 4.1).

Using a Canon EOS 60D reflex camera coupled to a Zeiss Discovery V8 stereo microscope by a macro lens (17-70 mm/F2.8-4.5), 25-minute recordings of single Collembola with direct contact to the volatile source were carried out. With 20 replicates per treatment, the recordings were made on several days with the same number of observations per treatment per day. Petri dishes (3 cm in diameter), filled with 5 ml water agar, were used as experimental arenas. Before start of a recording a new fungal colony or fungal-free medium control was placed in the centre of the arena and an animal was released outside of the colony. The wounding treatment in case of filamentous fungi was done immediately before recording using a thin wire loop. Nearly 50% of the fungal tissue were disrupted by scratching (two horizontal and two vertical scratches). During recording the arena was covered with a lid.

FITNESS RESPONSE TO FUNGAL DIETS (YEASTS AND FILAMENTOUS FUNGI)

To test whether the decisions made by Collembola during the searching and contact phase of foraging are reflected in fitness parameters, single Collembola were confronted with fungal colonies of *C. terricola*, *M. pulcherrima*, *T. dulcitum*, *A. nidulans*, *T. harzianum*, and *P. expansum* for 12 days. In light of the finding that yeasts are more accepted as food source than filamentous fungi (Subsection 4.4.3), it is expected that growth (body size) and reproduction (number of eggs) is also higher in the presence of yeasts.

Fungi were grown on circular pieces of absorbent filter paper (5 mm in diameter), which were previously soaked with MEA medium. In case of *C. terricola*, *M. pulcherrima*, *T. dulcitum*, *A. nidulans*, and *P. expansum* the medium patches were inoculated with 10 μl spore (1000 conidia/μl) or cell suspension, and in case of *T. harzianum* 5 μl spore suspension (2000 conidia/μl) were used for inoculation. Fungi were incubated for different time periods due to differences in growth properties (Table 4.1). Experimental arenas were prepared as follows: polypropylene cups (25 mm in diameter, 7 mm in height) were filled with a mixture of plaster and activated charcoal (proportion 40:1; 4 ml/cup). After hardening, the mixture was moistened with sterile water to prevent Collembola from drying out. One *F. candida* individual and one food patch (fungal colony or control patch) were added to the arena, which was then covered with fine mesh gauze and a lid with two air openings. Fungal-free MEA and nutrient-poor water agar served as controls. During the experimental period the substrate was moistened every 3 days and food patches were replaced once after 6 days before old colony patches were totally consumed. On the 6th and 12th day eggs were removed from arenas and counted. The body size of each animal was determined on the first and 12th day by means of a stereo microscope (Zeiss V8, Germany) and the computer software AxioVision (Zeiss, Germany). Animals that did not survive until the 12th day were excluded from the analysis. Each dietary treatment was replicated 15 times, viz. with respect to six fungal treatments and two control treatments, overall 120 samples were prepared.

4.3.6 SELECTION OF VARIABLES FOR CHARACTERISATION OF COLLEMBOLA FORAGING RESPONSES

Table 4.2: *Subject of the present study and overview on components of the food selection process (adapted from* Dethier et al. *(1960),* Schoonhoven et al. *(2005), and Chapter 2). By assuming that the here tested fungal colonies emit volatiles that Collembola can use as cues for food selection, the volatile designations (third column) refer to behavioural responses of animals characterised by the measured variables (second column).*

Food-selection phase	*Variables measured*	*Volatile designation*	*Null hypothesis*
Searching (off-patch) Location of potential fungal food sources	(1) Latency to the first arrival at the 'central zone'	Attractant	Collembola movement patterns are neither affected by the type of the fungal food source (fungal species, life form: yeast, filamentous microfungus) nor by fungal tissue wounding
		Repellent	
	(2) Residence time in the 'central zone' (3) Mean duration of 'central zone' visits (4) Duration of the first 'central zone' visit (5) Distribution of the residence time over the zones	Arrestant	
Contact (on-patch) Evaluation of potential fungal food souces	(6) Latency to the first colony contact (7) Latency to the first feeding event (8) Duration of the first contact event (9) Duration of the first feeding event (10) Number of colony contacts prior to the first feeding event (11) Contact time (12) Feeding time	Phagostimulant (acceptance)	Collembola contact behaviour and acceptance of fungal colonies as food source is neither affected by the type of fungus (fungal species, life form: yeast, filamentous microfungus) nor by wounding-related changes in fungal chemistry
		Deterrent (rejection)	

SEARCHING PHASE: OFF-PATCH MOVEMENT PATTERNS

Recordings were analysed with the semi-automated tracking software EthoVision® XT (version 8.0; Noldus Information Technology, Wageningen, Netherlands) (Noldus *et al.* 2001). To get detailed information on Collembola movement patterns, the position of individual animals was tracked every 0.52 seconds in relation to a 'central zone' that was defined around the volatile source by means of the arena settings in EthoVision® XT. The remaining space of the arena was further divided into five zones (Figure 4.1).

Following variables were selected to reveal attraction, avoidance, and/or arrestance: (1) 'latency to the first arrival at the central zone', (2) 'duration of the first central zone visit', (3) 'residence time in the central zone', (4) 'mean duration of central zone visits' (Table 4.2). If Collembola arrive faster or slower at the 'central zone' in comparison to the fungal-free control, they are assumed to be attracted or deterred respectively. A volatile source is assumed to have an arresting effect if the first 'central zone' visit is prolonged, the overall time spent in the 'central zone', and/or the 'mean duration of central zone visits' is longer in relation to the control. In addition, the 'distribution of residence time' over the predefined zones as a concentration gradient dependent variable was analysed. A deterrent effect of the volatile source is assumed if Collembola spent more time in outer zones of the experimental arena.

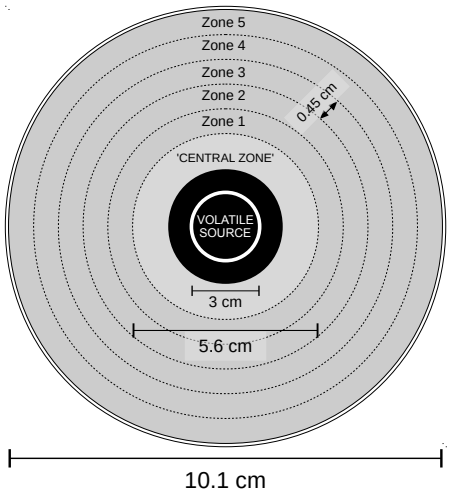

Figure 4.1: *Dimensions of the experimental arena. A 'central zone' was defined around the volatile source. Background noise and disruptions of the automated tracking process in EthoVision® XT were avoided by subtracting the area that encloses the volatile source (highlighted in black) from the arena by means of the 'arena settings'.*

CONTACT PHASE: ON-PATCH BEHAVIOUR

The computer software Observer® XT (version 10.0, Noldus Information Technology BV, Wageningen, The Netherlands) (Noldus 1991) was used to analyse the video footage. To get detailed information on Collembola contact and feeding behaviour following behavioural components were evaluated: latency to the first contact and first feeding event, duration of the first feeding event, total contact and feeding time, and contact frequency prior to the first feeding event (regarding the time interval between start of observation and onset of

feeding) (Table 4.2). In Chapter 2 these variables were demonstrated to be well suited for pointing out differences in the acceptance of fungal food sources by Collembola.

While the first contact with a patch can be assumed to be volatile-driven, subsequent behavioural responses of Collembola can be expected to be additionally driven by tactile and/or gustatory stimuli. Thus, if Collembola get in contact with a fungal colony more rapidly compared to controls, it is assumed that Collembola are attracted by the volatile bouquet of the respective fungus. If a fungus has an arresting effect, the animals are expected to spend a higher proportion of the observation time in contact compared to controls. Higher acceptance of a fungus as food source is assumed if Collembola start feeding faster and/or spend more time with feeding compared to the control. Contact with the resource patch was assumed when a part of the animal's body overlaps with the patch. Feeding activity was identified by short and jerky body movements and rhythmical up and down movements of the head while remaining stationary.

4.3.7 STATISTICAL ANALYSIS

GC-MS quantity data (manually integrated peak areas) of volatile compounds were compared between unwounded and wounded colonies of filamentous fungi using the Wilcoxon-Mann-Whitney test (α=0.05, distribution='exact', function 'wilcox_test', package 'coin' in R) to reveal wounding-related changes in volatile profiles. Compounds occurring in blank samples were fully removed from the data set and medium-derived compounds were not included in the statistical analysis.

The Wilcoxon test was also applied to determine the effect of fungal life-form (yeasts vs. filamentous fungi) and tissue wounding (unwounded vs. wounded) on the 'residence time in the central zone', the 'mean duration of central zone visits' (searching phase), and the 'contact time' (contact phase). On fungal species level, these variables were analysed using the non-parametric Kruskal-Wallis test (function 'kruskal.test') for more than two treatment groups. In case of global significance, pairwise comparisons were done using Dunn's post hoc test (Dunn 1964) (function 'dunnTest', 'dunn.test.control'; package 'FSA') with Benjamini-Hochberg correction of p-values. When ties were present values were ranked randomly by using the 'rank' function in R before applying post hoc tests. In case of non-parametric statistics effect size is represented by Cohen's d. As recording times slightly

differed with respect to the observation of the contact behaviour, the variables 'contact time' and 'feeding time' are given as percentage of the total observation time.

Cox regression models (function 'Coxph', package 'survival' in R) were applied on time dependent event data concerning the 'latency to the first arrival at the central zone', the 'duration of the first central zone visit' (searching phase), the 'latency to the first contact', the 'duration of the first contact', and the 'latency to the first feeding event' (contact phase). The assumption of proportionality was tested with goodness-of-fit $\tilde{\chi}^2$ tests along with an examination of partial residual plots (Schoenfeld 1980; 1982). As the 'duration of the first central zone visit' and the 'duration of the first contact' were intended to represent departure tendencies of Collembola, censoring was applied on 'visits' and 'contacts' that exceeded the observation time.

A generalized linear mixed effects model (function 'glmer', package 'lme4') with gamma distribution (log link function), fixed-effects of 'zone' and 'fungal species', and 'observation number' modelled as a random effect was applied to test for differences in the 'distribution of residence time' over the zones of the experimental arena between volatile sources (searching phase). As zeros were present within the continuous time data set, a value of 0.1 was added to each experimental value to meet the requirements of a gamma model. An analysis of variance (function 'Anova', package 'car') was applied on the mixed effects model to test for a significant global effect of the volatile source treatment.

The effect of fungal diets ('treatment') on the fitness parameter 'number of eggs' with zero-inflated and overdispersed count data was tested with a hurdle negative binomial regression model with 'logit' link function. In advance to this analysis an extensive model diagnosis was conducted, based on comparisons between possible error distributions, poisson or negative binomial, with respect to both zero-inflated (function 'zeroinfl', package 'pscl') and hurdle models (function 'hurdle', package 'pscl') by visual examination of diagnostic plots (residuals, Cook's D) and comparison of AIC and BIC values. Neither inclusion of the parameter 'growth' (change in body length) in the negative binomial part (positive count process) nor inclusion of 'treatment' in the truncated binomial part (zero count process) improved fit of the hurdle model, therefore both variables were excluded from the respective parts of the model. The final model was determined as follows: 'hurdle(no.eggs treatment | growth.x, link = "logit", dist = "negbin")' (the | separates the count model from the logistic model). Global significance was tested using the Wald test (function 'waldtest').

Analysis of the fitness parameter 'growth' (change in body length) in response to fungal

dietary treatments was done using a generalized linear model with gamma error distribution and 'log' link function (function 'glm' in R). A likelihood ratio test was applied to test for global significance. Correlation between fitness parameters 'number of eggs' and 'change in body length' was tested with Pearson's product-moment correlation test (function 'cor.test').

4.4 RESULTS

4.4.1 GC-MS ANALYSIS - COMPARISON OF VOLATILE PROFILES

Overall, 89 volatile compounds were determined from headspace samples of *C. terricola*, *M. pulcherrima*, and *T. dulcitum* yeasts, and vegetative and sporulating, unwounded and wounded colonies of *A. nidulans* (two genotypes), *T. harzianum*, and *P. expansum* (two genotypes) (Table 4.1) (Table A.4.2), after deduction of MEA medium derived compounds and artefacts of the chromatographic column. With regard to the presence and absence of these 89 compounds, striking differences were found between yeasts and filamentous fungi. With only 7 compounds, considerably less compounds where detected in yeast samples compared to filamentous fungi from which 84 compounds were determined. Whereas only alcohols and acetates were identified from yeast samples, the volatile profiles of the three filamentous fungi comprise alcohols, alkenes, aromatic compounds, ketones, terpenes, sesquiterpenes, and sesquiterpenoids (Table A.4.2).

Compound overlap between the two fungal life forms were found in case of 2-methyl-1-propanol which was detected in samples of the yeast *M. pulcherrima* as well as in samples of filamentous fungi *T. harzianum*, and *P. expansum*, and in case of 3-methyl-1-butanol that was emitted by all species, genotypes and growth stages, except from sporulating colonies of *T. harzianum* (Table A.4.2).

Out of the three yeasts *M. pulcherrima* was the richest in volatiles with 3-octanol acetate, isoamyl acetate, isobutyl acetate, 2-methyl-1-propanol, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol, and phenylethyl alcohol. Only one compound, 3-methyl-1-butanol, was also detected in *C. terricola* and *T. dulcitum* (Table A.4.2).

Similarly, only few compounds were detected in samples of the filamentous fungus *A. nidulans*. Whereas three compounds, 3-methyl-1-butanol, 3-octanone, and 1-octen-3-ol, were found in the headspace of the vegetative growth stage of the VeA1 genotype, only 3-methyl-1-butanol was detected in sporulating colonies of this genotype (Table A.4.2). With respect to the vegetative stage 3-octanone (Wilcoxon-Mann-Whitney test, z=-2.21, p=0.028) and 1-octen-3-ol (Wilcoxon-Mann-Whitney test, z=-2.21, p=0.028) quantities significantly increased in response to wounding (Table A.4.3). Five further compounds were found to be wound-activated (Table 4.3). In case of the sporulating growth stage of *A. nidulans* seven compounds were found to be activated by wounding in the VeA1 genotype and six wound-activated compounds were identified from the VeA+ genotype (Table 4.3). The sporulating growth stage of the VeA1 genotype had been selected for further experiments.

Unlike *A. nidulans*, a large set of terpenes was detected in samples of filamentous fungi *T. harzianum* and *P. expansum*. Sporulating colonies of these species differentiate from vegetative colonies mainly by a larger number of terpenes (Table A.4.2).

Among all tested fungi, *P. expansum* was characterised by the emission of geosmin, 2-methyl-isoborneol, and it's dehydration products 2-methyl-2-bornene and 2-methylenebornane (Martin *et al.* 1988a) (Table A.4.2). Overall, 33 compounds were detected in sporulating colonies of *P. expansum* genotype A and 29 compounds were found to derive from genotype B; thus, the sporulating growth stage of genotype A was selected for further experiments. With respect to this genotype, six compounds were found to be wound-activated (Table 4.3).

The volatile profile of the sporulating growth stage of *T. harzianum* comprised the largest number of volatile compounds among all tested fungi, in total 44 compounds (Table A.4.2). Whereas four compounds were only detected in samples of wounded colonies, abundances of the alcohol 1-octen-3-ol (Wilcoxon-Mann-Whitney test, z=-2.93, p=0.002) and tentatively identified sesquiterpenes β-funebrene (Wilcoxon-Mann-Whitney test, z=-2.08, p=0.041) and nardosina-9,11-diene (Wilcoxon-Mann-Whitney test, z=-2.40, p=0.015) as well as abundances of an unidentified compound ('unknown (26)') (Wilcoxon-Mann-Whitney test, z=-2.08, p=0.041) were increased in response to fungal tissue wounding (Table 4.3, Table A.4.3).

Table 4.3: *Overview of wound-activated volatile compounds that were exclusively found in headspace samples of wounded colonies (⋆), increased (▲), or no longer detected (○).*

	A. nidulans				*P. expansum*				*T. harzianum*	
	VeA1		VeA+		A		B			
	veg	spo	veg	spo	veg	spo	veg	spo	veg	spo
1-octen-3-ol	▲	⋆	▲	⋆	▲	⋆	⋆	⋆	⋆	▲
1-octen-3-ol acetate	⋆	⋆	⋆	⋆						
1,3-octadiene	⋆	⋆	⋆	⋆	⋆	⋆	⋆	⋆	⋆	⋆
1,3-trans-5-trans-octatriene	⋆	⋆	⋆	⋆	⋆	⋆	⋆	⋆		⋆
2,4,6-octatriene	⋆	⋆	⋆	⋆	⋆	⋆	⋆	⋆		⋆
3,3,5-trimethyl-2-hexene	⋆	⋆	⋆	⋆	⋆	⋆	⋆	⋆		
3-octanone	▲	⋆	⋆		▲	⋆	⋆	⋆		⋆
2-methyl-1-propanol					○					
2-methylenebornane								▲		
trans-β-bergamotene									▲	
β-funebrene										▲
Nardosina-9,11-diene										▲
Unknown (26)										▲

4.4.2 THE SEARCHING PHASE OF FOOD SELECTION

EFFECT OF FUNGAL SPECIES

With focus on the response of *F. candida* to fungal species, significant global effects were found with respect to the 'latency to the first arrival at the central zone' (arrival tendency) (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=15.29, df=6, p=0.018) and the 'residence time in the central zone' (Kruskal-Wallis test, $\tilde{\chi}^2$=19.38, df=6, p=0.004). Relative to the control group the tendency to arrive at 'central zones' of *P. expansum* colonies was significantly lower and only half as high with a hazard ratio of 0.49, however, no significant differences were found between the control treatment and the fungal species *A. nidulans*, *T. harzianum*, *C. terricola*, *M. pulcherrima*, and *T. dulcitum* (Table 4.4, Figure 4.2). The 'residence time in the central zone' of the yeast *C. terricola* was significantly higher compared to controls (Table 4.5, Figure 4.3a). An overall effect of the fungal species was also observed with respect to the 'mean duration of central zone visits' (Kruskal-Wallis test, $\tilde{\chi}^2$=21.03, df=6, p=0.002) with a trend towards higher mean visit durations in the presence of *C. terricola* (140.04 sec, sd=183.77 sec) and the filamentous fungus *A. nidulans* (172.34 sec, sd=258.57 sec) compared to controls (51.79 sec, sd=97.32 sec) (Table 4.5). The tendency to leave the central zone subsequent to the first

central zone visit ('duration of the first central zone visit') (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=3.2, df=6, p=0.784) and the 'distribution of the residence time' over the predefined zones (Gamma regression, ANOVA Type II, $\tilde{\chi}^2$=1.80, df=6, p=0.937) were not affected by fungal species.

Table 4.4: *Searching phase: Cox regression test statistic for time-dependent variables 'latency to the first arrival at the central zone' (arrival tendency) and 'duration of the first central zone visit' (departure tendency) in response to filamentous fungi* A. nidulans *(VeA1),* T. harzianum*, and* P. expansum *(A), and yeasts* C. terricola*,* M. pulcherrima*, and* T. dulcitum. $\exp(coef)^{(*)}$ *gives the risk of the respective event in relation to the MEA control group.*

Species	*Behavioural variable*	n	*Events*	Z	$\exp(coef)^{(*)}$	$P-value$
MEA control	First arrival	30	26			
A. nidulans	First arrival	30	25	-0.74	0.81	0.457
T. harzianum	First arrival	30	26	-0.14	0.96	0.892
P. expansum	First arrival	30	20	-2.38	0.49	**0.017**
C. terricola	First arrival	30	27	1.09	1.35	0.277
M. pulcherrima	First arrival	30	29	0.60	1.18	0.548
T. dulcitum	First arrival	30	27	-0.12	0.97	0.902
MEA control	Duration first visit	26	25			
A. nidulans	Duration first visit	25	23	-1.47	0.65	0.143
T. harzianum	Duration first visit	26	24	-1.10	0.73	0.270
P. expansum	Duration first visit	20	19	-0.78	0.79	0.434
C. terricola	Duration first visit	27	26	-1.24	0.70	0.216
M. pulcherrima	Duration first visit	29	25	-1.40	0.67	0.163
T. dulcitum	Duration first visit	27	25	-0.52	0.86	0.604

$^{(*)}$ With respect to the 'first arrival' a value greater than 1 indicates higher arrival tendency in a treatment group compared to the control, and a value smaller than 1 indicates a lower arrival tendency, respectively. Regarding the 'duration of the first visit' a value smaller than 1 indicates higher tendency for remaining (lower departure tendency) in the central zone of a fungal treatment, compared to the control group.

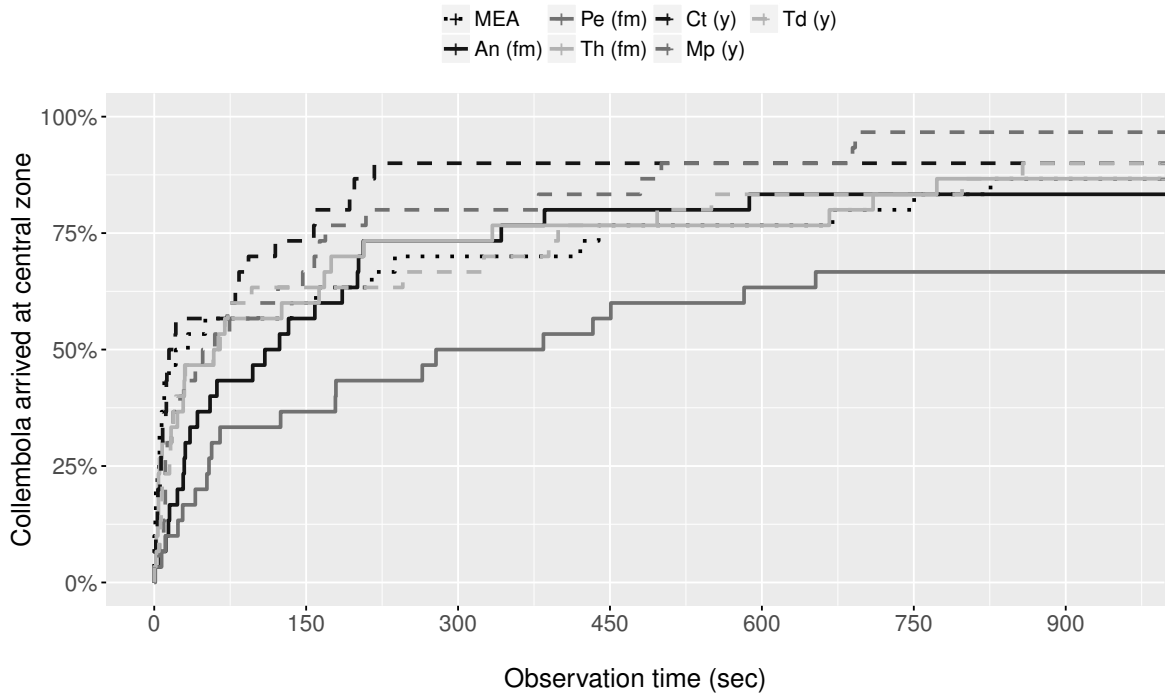

Figure 4.2: *Kaplan Meier curves of* F. candida *'first arrival at the central zone' in response to filamentous fungi (fm)* A. nidulans *(An),* T. harzianum *(Th), and* P. expansum *(Pe), yeasts (y)* C. terricola *(Ct),* M. pulcherrima *(Mp), and* T. dulcitum *(Td), and MEA controls (MEA) within an observation period of 15 minutes.*

Table 4.5: *Searching phase: test statistic of pairwise comparisons using Dunn's test with respect to the 'residence time in the central zone' and the 'mean duration of central zone visits' in response to fungal species. In case of same sample size the effect size is represented by Cohen's d and in case of different sample size Hedge's g is given, with 95% confidence interval.*

Behavioural variable	*Comparison*	N	Z	$Effect\ size\ (CI)$	$P-value$	$^{(*)}$
Time in zone	Control - *A. nidulans*	60	1.33	0.48 (-0.05, 1.00)	0.237	
Time in zone	Control - *T. harzianum*	60	1.33	0.41 (-0.11, 0.93)	0.237	
Time in zone	Control - *P. expansum*	60	1.08	-0.14 (-0.66, 0.38)	0.280	
Time in zone	Control - *C. terricola*	60	2.87	0.90 (0.36, 1.44)	**0.024**	▲
Time in zone	Control - *M. pulcherrima*	60	1.89	0.57 (0.04, 1.10)	0.178	
Time in zone	Control - *T. dulcitum*	60	1.29	0.42 (-0.11, 0.94)	0.237	
Mean visit duration	Control - *A. nidulans*	51	2.23	0.61 (0.04, 1.19)	0.075	
Mean visit duration	Control - *T. harzianum*	52	1.80	0.49 (-0.07, 1.06)	0.143	
Mean visit duration	Control - *P. expansum*	46	1.42	0.17 (-0.43, 0.77)	0.233	
Mean visit duration	Control - *C. terricola*	53	2.40	0.59 (0.02, 1.15)	0.075	
Mean visit duration	Control - *M. pulcherrima*	55	0.81	0.51 (-0.04, 1.06)	0.421	
Mean visit duration	Control - *T. dulcitum*	53	0.93	0.30 (-0.26, 0.85)	0.420	

$^{(*)}$ Referred to the second entry in the column 'Comparison' ▲ indicates a significant increase in the respective variable.

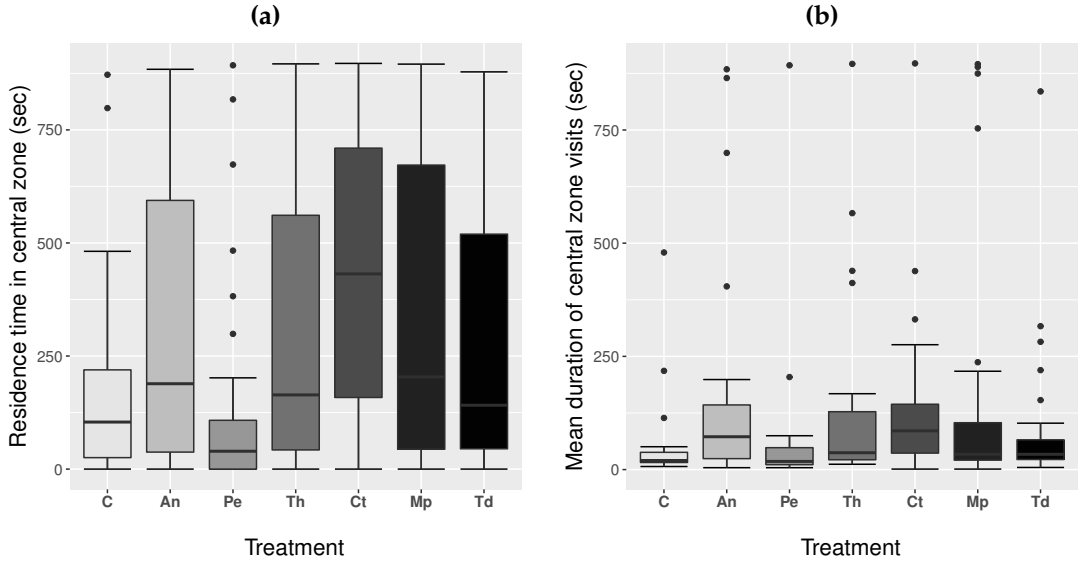

Figure 4.3: F. candida ***(a)*** *'residence time in the central zone' and* ***(b)*** *'mean duration of central zone visits' in response to filamentous fungi* A. nidulans *(An),* T. harzianum *(Th), and* P. expansum *(Pe), yeasts* C. terricola *(Ct),* M. pulcherrima *(Mp), and* T. dulcitum *(Td), and MEA controls (C) within an observation period of 15 minutes.*

FILAMENTOUS FUNGI VS. YEASTS

Analysis of Collembola responses to yeasts and filamentous fungi (pooled data) revealed significant effects of the fungal life form on the 'latency to the first arrival at the central zone' and the 'residence time in the central zone' (Table 4.6). The tendency to arrive at the 'central zone' was 1.6 times higher in the presence of yeasts and Collembola spent significantly more time in the 'central zone' in the presence of yeasts compared to filamentous fungi. The tendency to leave the patch after the first 'central zone' visit ('duration of the first central zone visit'), the 'mean duration of central zone visits', and the 'distribution of the residence time' over the predefined zones were not affected by the fungal life form (Table 4.6, Figure 4.4). However, when the most attractive yeast, *C. terricola*, and the most unattractive filamentous fungus, *P. expansum*, were excluded from the analysis, the effect of the fungal group on both the arrival tendency (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=1.08, df=1, p=0.299) and residence time (Wilcoxon-Mann-Whitney test, z=-0.37, df=1, p=0.713) was not significant anymore.

Table 4.6: *Searching phase: test statistic for 'latency to the first arrival at the central zone' (arrival tendency), 'duration of the first central zone visit' (departure tendency) (Cox regression), 'residence time in the central zone', 'mean duration of central zone visits' (Wilcoxon test), and 'distribution of the residence time' (Gamma regression, ANOVA Type II) in response to fungal life forms (yeasts vs. filamentous fungi, pooled data). For Cox and Gamma regression models the $\tilde{\chi}^2$ value and for Wilcoxon tests the Z-score is given. Effect size is represented by the hazard ratio ($\exp(coef)^{(*)}$) and Cohen's d or Hedge's g, with 95% confidence interval.*

Behavioural variable	N	*Events*	Df	$\tilde{\chi}^2/Z$	$Effect\ size\ (CI)$	$P-value$	$^{(*)}$
First arrival	180	154	1	8.34	1.60	**0.004**	▲
Duration first visit	154	142	1	0.03	1.03	0.872	
Time in zone	180		1	2.57	0.35 (0.05, 0.64)	**0.010**	▲
Mean visit duration	154		1	0.80	0.01 (-0.31, 0.33)	0.428	
Distribution residence time	180		1	0.58		0.446	

Given effect sizes relate to the yeast group. With respect to the first arrival a value greater than 1 indicates higher arrival tendency in the yeast group compared to the filamentous fungi group, and a value smaller than 1 lower arrival tendency, respectively. Regarding the 'duration of the first central zone visit' a value smaller than 1 indicates higher tendency for remaining (lower departure tendency) in the yeast 'central zones' compared to filamentous fungi.

$^{(*)}$ With reference to the yeast group ▲ indicates a significant increase in the respective variable compared to filamentous fungi group.

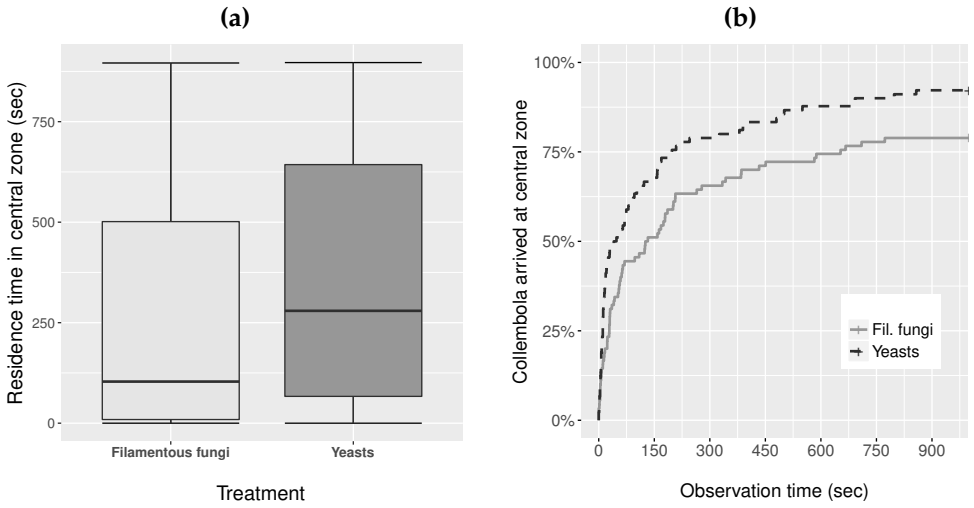

Figure 4.4: F. candida ***(a)*** *'residence time in the central zone' and* ***(b)*** *Kaplan Meier curves for the 'first arrival at the central zone' in response to fungal life forms (yeasts vs. filamentous fungi, pooled data) within an observation period of 15 minutes.*

UNWOUNDED VS. WOUNDED COLONIES OF FILAMENTOUS FUNGI

None of the examined variables was affected by the wounding treatment, neither in case of pooled data (Table 4.7) nor when focussing on fungal species *A. nidulans*, *P. expansum*, and *T. harzianum* separately (Table 4.8). However, there is a clear trend towards higher arrival tendencies in the presence of unwounded colonies (Table 4.7, Figure 4.5).

Table 4.7: *Searching phase: test statistic for 'latency to the first arrival at the central zone' (arrival tendency), 'duration of the first central zone visit' (departure tendency) (Cox regression), 'residence time in the central zone', and 'mean duration of central zone visits' (Wilcoxon test), and the 'distribution of the residence time' over zones (Gamma regression, ANOVA Type II) in response to unwounded and wounded colonies of filamentous fungi (pooled data). For Cox and Gamma regression models $\tilde{\chi}^2$ values and for Wilcoxon tests the Z-score is given. Effect size is represented by the hazard ratio ($\exp(coef)^{(*)}$) and Cohen's d or Hedge's g, with 95% confidence interval.*

Behavioural variable	N	*Events*	Df	$\tilde{\chi}^2/Z$	$Effect\ size\ (CI)$	$P-value$
First arrival	180	132	1	3.21	0.73	0.073
Duration first visit	132	122	1	0.62	0.87	0.432
Time in zone	180		1	-0.60	-0.07 (-0.37, 0.22)	0.549
Mean visit duration	132		1	0.65	-0.02 (-0.35, 0.31)	0.517
Distribution of time	180		1	0.03		0.863

Given effect sizes relate to the wounding treatment group. With respect to the first arrival a value greater than 1 indicates higher arrival tendency in the wounding treatment group, and a value smaller than 1 lower arrival tendency, respectively. Regarding the 'duration of the first central zone visit' a value smaller than 1 indicates higher tendency for remaining (lower departure tendency) in 'central zones' of wounded colonies.

Table 4.8: *Searching phase: test statistic for 'latency to the first arrival at the central zone' (arrival tendency), 'duration of the first central zone visit' (departure tendency) (Cox regression), 'residence time in the central zone', 'mean duration of central zone visits' (Wilcoxon test), and the 'distribution of the residence time' over the zones (Gamma regression, ANOVA Type II) in response to unwounded and wounded colonies of filamentous fungi (separate analysis per fungal species). For Cox and Gamma regression models the $\tilde{\chi}^2$ value and for Wilcoxon tests the Z-score is given. Effect size is represented by the hazard ratio ($\exp(coef)^{(*)}$) and Cohen's d or Hedge's g, with 95% confidence interval.*

Species	*Behavioural variable*	N	*Events*	Df	$\tilde{\chi}^2/Z$	$Effect\ size\ (CI)$	$P-value$
A. nidulans	First arrival	60	46	1	1.51	0.69	0.219
P. expansum	First arrival	60	37	1	0.33	0.83	0.567
T. harzianum	First arrival	60	49	1	2.04	0.66	0.154
A. nidulans	Duration first visit	46	43	1	0.11	1.11	0.741
P. expansum	Duration first visit	37	35	1	0.65	0.76	0.421
T. harzianum	Duration first visit	49	44	1	0.69	0.78	0.407
A. nidulans	Time in zone	60		1	0.01	0.13 (-0.39, 0.65)	0.991
P. expansum	Time in zone	60		1	-0.27	0.01 (-0.51, 0.52)	0.789
T. harzianum	Time in zone	60		1	-0.53	0.15 (-0.66, 0.37)	0.601
A. nidulans	Mean visit duration	46		1	0.39	-0.08 (-0.68, 0.51)	0.710
P. expansum	Mean visit duration	37		1	0.55	0.03 (-0.63, 0.70)	0.598
T. harzianum	Mean visit duration	49		1	0.40	0.05 (-0.53, 0.62)	0.699
A. nidulans	Distribution of time	60		1	0		1.000
P. expansum	Distribution of time	60		1	0.08		0.776
T. harzianum	Distribution of time	60		1	0.02		0.896

Given effect sizes relate to the wounded treatment group.

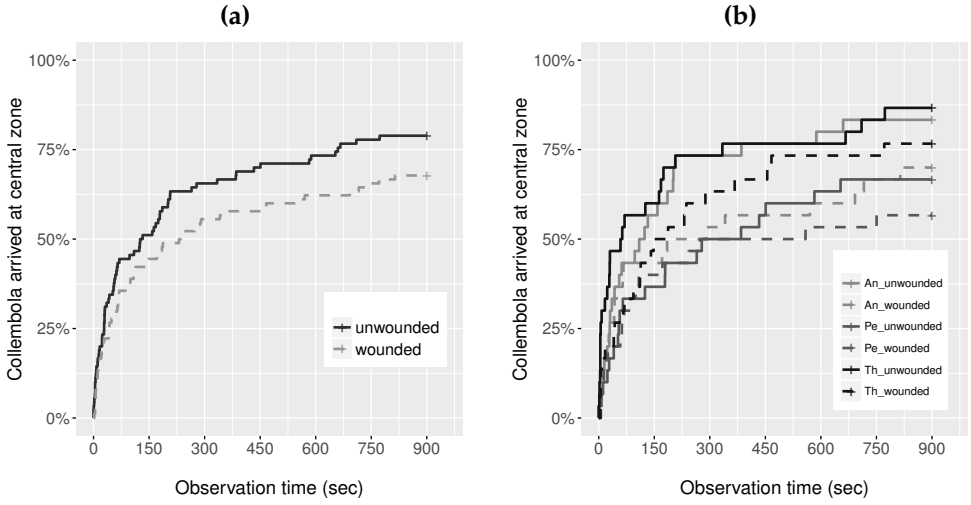

Figure 4.5: *Kaplan-Meier curves for* F. candida *first arrival at the 'central zone' in response to unwounded and wounded colonies of filamentous fungi within an observation period of 15 minutes. Presentation on* ***(a)*** *treatment level (pooled data across species) and* ***(b)*** *species level (An: A. nidulans; Pe: P. expansum; Th: T. harzianum.*

COLLEMBOLA RESPONSES TO GEOSMIN

Collembola movement patterns were affected by the *P. expansum* characteristic volatile compound geosmin. Significant global effects were found with respect to the 'first arrival at the central zone' (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=12.10, df=2, p=0.002) and the 'residence time in the central zone' (Kruskal-Wallis test, $\tilde{\chi}^2$=8.80, df=2, p=0.012). With an inverted hazard ratio of 4.33 (1/0.23) Collembola arrival tendency was more than four times higher in the presence of the solvent control than the tendency in presence of the high concentration of geosmin (Table 4.9, Figure 4.6). Further, Collembola spent significantly less time in the 'central zone' of the high concentration of geosmin compared to controls (Table 4.10, Figure 4.6). In contrast, the time spent in the 'central zone' of the low concentration of geosmin was not different to controls, but significantly higher compared to the high concentration of geosmin (Table 4.10). A global effect of the geosmin treatment was also found with respect to the 'distribution of residence time' over the predefined zones (Gamma regression, ANOVA Type II, $\tilde{\chi}^2$=13.76, df=2, p=0.001). The distribution did not differ from the solvent control in case of the low concentration of geosmin (t=0.86, p=0.390), but a significant difference was found between the control and the high concentration of geosmin (t=-2.75, p=0.006). Whereas Collembola spent the majority of the observation time in outer

zones, most notably in the border zone of the arena, and hardly in zones close to the volatile source ('central zone', 'zone 1'), in presence of the solvent control the residence time was distributed more evenly over the zones with significant residence also in zones close to the volatile source (Figure 4.7). The 'duration of the first central zone visit' (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=0.09, df=2, p=0.956), and the 'mean duration of central zone visits' were not affected by geosmin (Kruskal-Wallis test, $\tilde{\chi}^2$=0.61, df=2, p=0.731).

Table 4.9: *Searching phase: test statistic for 'latency to the first arrival at the central zone' (arrival tendency), 'duration of the first central zone visit' (departure tendency) in response to the* P. expansum *characteristic compound geosmin.*

Treatment	*Behavioural variable*	n	*Events*	Z	$\exp(coef)^{(*)}$	$P-value$	$^{(**)}$
Solvent control	First arrival	20	10				
Geosmin 10^{-3}	First arrival	20	3	-2.22	0.23	**0.026**	▽
Geosmin 10^{-5}	First arrival	20	13	1.02	1.53	0.309	
Solvent control	Duration first visit	10	8				
Geosmin 10^{-3}	Duration first visit	3	2	-0.24	0.83	0.812	
Geosmin 10^{-5}	Duration first visit	13	10	-0.25	0.89	0.800	

$^{(*)}$ With respect to the first arrival a value greater than 1 indicates higher arrival tendency in a treatment group in comparison to the control, and a value smaller than 1 lower arrival tendency, respectively. Regarding the 'duration of the first central zone visit' a value smaller than 1 indicates higher tendency for remaining (lower departure tendency) in the 'central zone' in the presence of geosmin compared to the control group.
$^{(**)}$ Compared to the control group ▽ indicates a significant decrease in the respective variable.

Table 4.10: *Searching phase: test statistic of pairwise comparisons using Dunn's test for multiple comparisons of means with respect to the 'residence time in the central zone' in response to geosmin. Effect size is represented by Cohen's d, with 95% confidence interval.*

Variable	*Comparison*	N	Z	$Effect\ size\ (CI)$	$P-value$	$^{(*)}$
Time in zone	Control - Geosmin 10^{-3}	40	-2.47	-0.22 (-0.86, 0.42)	**0.020**	▽
Time in zone	Control - Geosmin 10^{-5}	40	1.21	0.30 (-0.35, 0.94)	0.227	
Time in zone	Geosmin 10^{-3} - Geosmin 10^{-5}	40	3.68	0.54 (-0.12, 1.19)	**<0.001**	▲

$^{(*)}$ Referred to the second entry in the column 'Comparison' ▲ indicates a significant increase and ▽ a significant decrease in the respective variable.

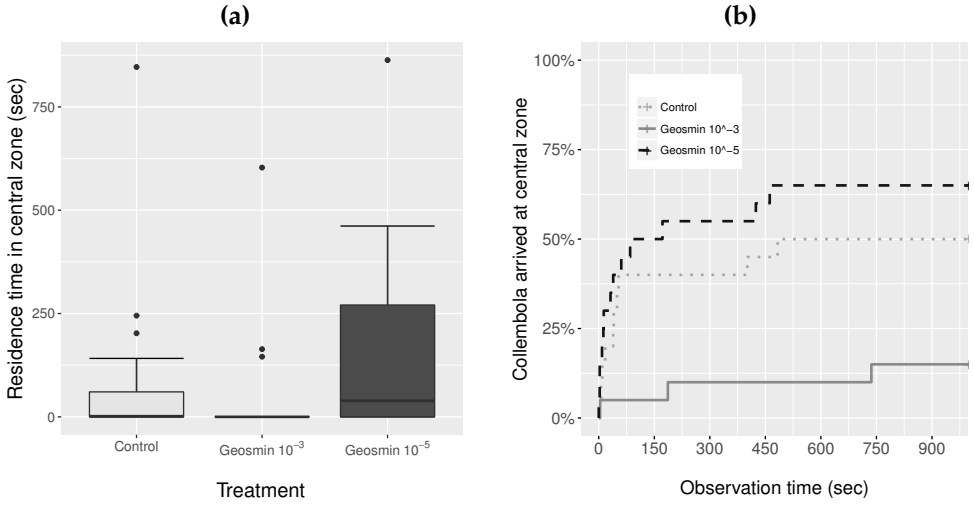

Figure 4.6: F. candida ***(a)*** *'residence time in the central zone' and* ***(b)*** *Kaplan Meier curves for the 'first arrival at the central zone' in response to the* P. expansum *characteristic compound geosmin (concentrations 10^{-3} and 10^{-5}) and solvent controls.*

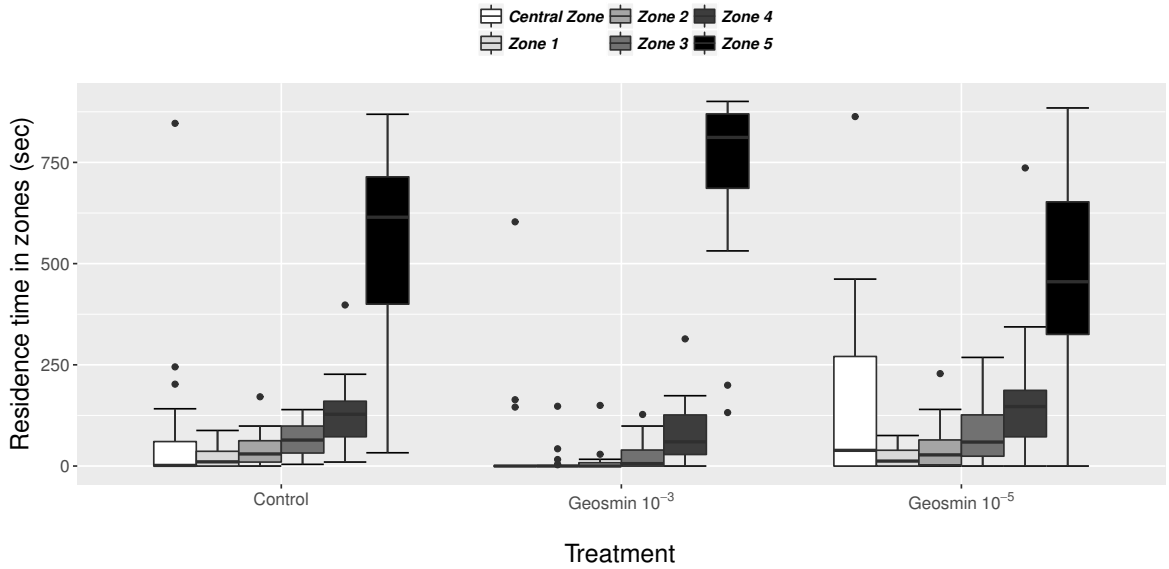

Figure 4.7: *'Distribution of residence time' over predefined zones in response to the* P. expansum *characteristic compound geosmin (concentrations 10^{-3} and 10^{-5}) and solvent controls.*

4.4.3 CONTACT BEHAVIOUR OF *F. candida*

EFFECT OF FUNGAL SPECIES

A significant global effect of fungal species on the contact behaviour of *F. candida* was found with respect to the 'latency to the first contact' (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=21.10, df=6, p=0.002), the 'duration of the first contact' (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=13.38, df=6, p=0.037), and the 'contact time' (Kruskal-Wallis test, $\tilde{\chi}^2$=32.94, df=6, p<0.001). Pairwise comparisons against the control treatment revealed that Collembola contact tendencies ('latency to the first contact') were about 2.6 times higher in the presence of *M. pulcherrima* and *T. dulcitum* yeast fungal colonies (Table 4.11, Figure 4.8a). In line with this, Collembola spent a significantly higher percentage of the observation time in contact with *T. dulcitum* colonies compared to MEA controls (Table 4.12, Figure 4.9). Furthermore, the tendency to leave a *T. dulcitum* colony after having the first contact was less than half as high as the tendency to leave a control patch, viz. the tendency to stay in further contact with a *T. dulcitum* colony was considerably higher (Figure 4.8a).

The 'latency to the first feeding event' was significantly affected by the fungal treatment (Cox regression, likelihood ratio test, $\tilde{\chi}^2$=15.31, df=6, p=0.018), but in general the feeding activity was low. The highest number of feeding events was observed in the presence of *T. dulcitum*; 9 out of 20 Collembola fed on *T. dulcitum* colonies. Only one individual was observed to feed on colonies of filamentous fungi *P. expansum* and *T. harzianum*, respectively (Figure 4.8b), with a negligible percentage of the observation time spent with feeding. However, as comparisons of feeding tendencies against the control were not statistically significant (Table 4.11) results only represent trends towards a higher feeding tendency in the presence of *T. dulcitum* and lower feeding tendencies in response to *P. expansum* and *T. harzianum*.

Due to the low number of feeding events in the presence of *P. expansum* and *T. harzianum*, an appropriate statistical analysis of the variables 'first feeding duration' (tendency to interrupt feeding), 'time feeding', and 'patch contact frequency prior feeding' was not possible. The corresponding raw data table is included in the appendix (Table A.4.4).

Table 4.11: *Contact phase: Cox regression test statistic for the time-dependent variable 'latency to the first contact' (contact tendency), 'duration of the first contact' (departure tendency), and 'latency to the first feeding event' in response to fungal species.* $\exp(coef)^{(*)}$ *gives the risk of the respective event in comparison to the MEA control group.*

Species	*Behavioural variable*	n	*Events*	Z	$\exp(coef)^{(*)}$	$P-value$	$^{(**)}$
MEA control	First contact	20	12				
A. nidulans	First contact	20	14	1.02	1.49	0.308	
T. harzianum	First contact	20	11	-0.59	0.78	0.553	
P. expansum	First contact	20	11	-0.50	0.81	0.621	
C. terricola	First contact	20	15	1.16	1.57	0.248	
M. pulcherrima	First contact	20	17	2.54	2.62	**0.011**	▲
T. dulcitum	First contact	20	18	2.60	2.66	**0.009**	▲
MEA control	First contact duration	12	12				
A. nidulans	First contact duration	14	12	-1.49	0.54	0.136	
T. harzianum	First contact duration	11	11	0.23	1.10	0.824	
P. expansum	First contact duration	11	11	1.03	1.55	0.305	
C. terricola	First contact duration	15	15	-1.26	0.61	0.207	
M. pulcherrima	First contact duration	17	16	-0.94	0.70	0.348	
T. dulcitum	First contact duration	18	17	-2.17	0.44	**0.030**	▽
MEA control	First feeding	20	4				
A. nidulans	First feeding	20	6	0.62	1.49	0.534	
T. harzianum	First feeding	20	1	-1.36	0.22	0.174	
P. expansum	First feeding	20	1	-1.34	0.22	0.182	
C. terricola	First feeding	20	4	-0.004	1.00	0.997	
M. pulcherrima	First feeding	20	6	0.62	1.49	0.537	
T. dulcitum	First feeding	20	9	1.54	2.53	0.174	

$^{(*)}$ Ratio of hazard rates between two groups. With respect to the latency to the first contact and feeding event a hazard ratio greater than 1 indicates higher contact and/or feeding tendency in a treatment group relative to the control, and a value smaller than 1 lower contact and/or feeding tendency, respectively. Regarding the 'duration of the first contact' a value smaller than 1 indicates higher tendency for remaining (i.e. lower departure tendency) in contact with the respective fungal source relative to the control group.

$^{(**)}$ Compared to the control group ▽ indicates a significant decrease and ▲ a significant increase in the respective variable.

Table 4.12: *Contact phase: test statistic of pairwise comparisons using Dunn's test for multiple comparisons of means with respect to the 'contact time' in response to fungal species. Effect size is represented by Cohen's d, with 95% confidence interval.*

Behavioural variable	*Comparison*	N	Z	$Effect\ size\ (CI)$	$P-value$	$^{(**)}$
Contact time	Control - *A. nidulans*	40	2.03	0.56 (-0.09, 1.21)	0.084	
Contact time	Control - *T. harzianum*	40	0.04	-0.55 (-1.20, 0.10)	0.819	
Contact time	Control - *P. expansum*	40	0.41	-0.46 (-1.11, 0.19)	0.969	
Contact time	Control - *C. terricola*	40	1.91	0.31 (-0.33, 0.96)	0.084	
Contact time	Control - *M. pulcherrima*	40	2.21	0.35 (-0.30, 0.99)	0.081	
Contact time	Control - *T. dulcitum*	40	4.20	0.94 (0.27, 1.62)	**<0.001**	▲

$^{(*)}$ Compared to the control group ▲ indicates a significant increase in the respective behavioural variable.

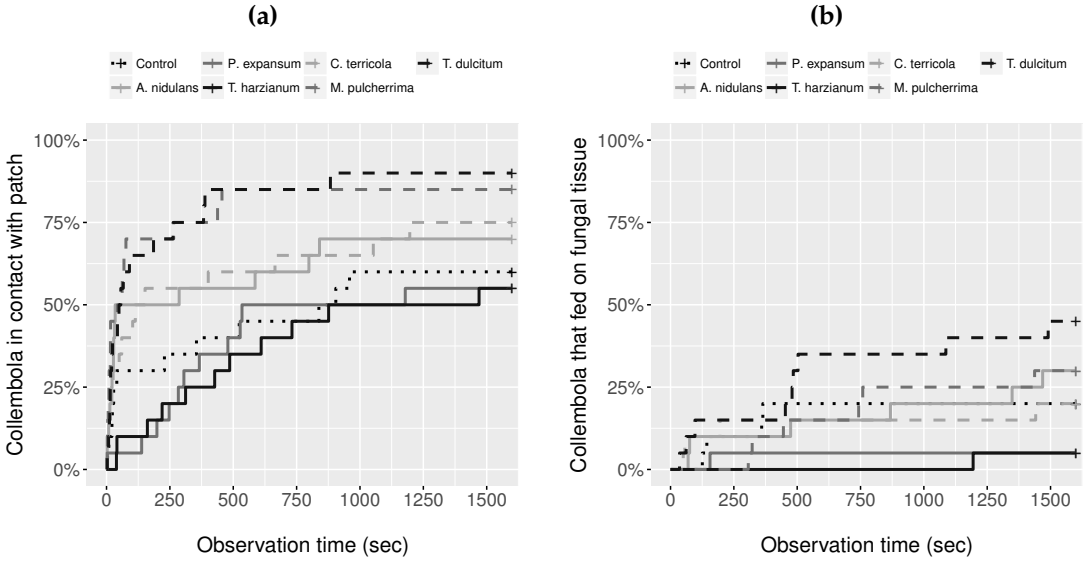

Figure 4.8: *Contact phase: Kaplan-Meier curves for* F. candida ***(a)*** *'latency to the first colony contact' (contact tendency) and* ***(b)*** *'latency to the first feeding event' (feeding tendency) in response to colonies of filamentous fungi, yeasts, and MEA controls within an observation period of ~25 minutes.*

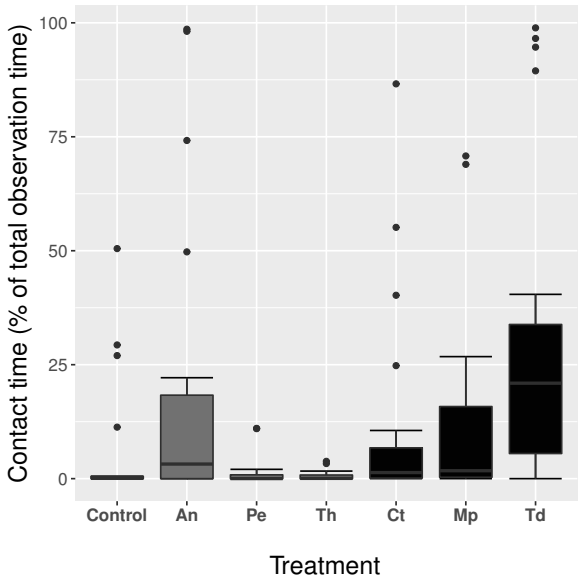

Figure 4.9: *Contact phase: 'contact time' of* F. candida *in response to colonies of filamentous fungi, yeasts, and MEA controls.*

FILAMENTOUS FUNGI VS. YEASTS

The comparison between fungal life forms revealed that the tendency to get in contact with yeast colonies was 2.24 times higher and the tendency to start feeding on yeasts was 2.68 times higher than the tendency to get in contact with and feed on filamentous fungi (Table 4.13, Figure 4.10). The proportion of the observation time Collembola stayed in contact with a yeast colony was also significantly higher compared to filamentous fungi (Table 4.13, Figure 4.11). No significant effect of the fungal life form was found with respect to the departure tendency ('duration of the first contact').

Table 4.13: *Contact phase: test statistic for 'latency to the first contact' (contact tendency), 'duration of the first contact' (departure tendency), 'latency to the first feeding event' (feeding tendency) (Cox regression), and 'contact time' (Wilcoxon test) in response to fungal life forms (yeasts and filamentous fungi; pooled data). For Cox regression models $\tilde{\chi}^2$ values, and for Wilcoxon tests the Z-score is given. Effect size is represented by the hazard ratio ($\exp(coef)^{(*)}$) and Cohen's d, with 95% confidence interval.*

Behavioural variable	N	*Events*	Df	$\tilde{\chi}^2/Z$	$Effect\ size\ (CI)$	$P-value$
First contact	120	86	1	13.56	2.24	**<0.001**
Duration first contact	86	82	1	3.22	0.66	0.073
First feeding	120	27	1	6.05	2.68	**0.014**
Contact time	120		1	-3.86	0.44 (0.08, 0.81)	**<0.001**

Given effect sizes relate to the yeast group. With respect to the latency to the first contact and feeding event a hazard ratio greater than 1 indicates higher contact and/or feeding tendency in the yeast group, and a value smaller than 1 lower contact and/or feeding tendency, respectively. Regarding the duration of the first visit a value smaller than 1 indicates higher tendency for remaining (i.e. lower departure tendency) in contact with yeasts.

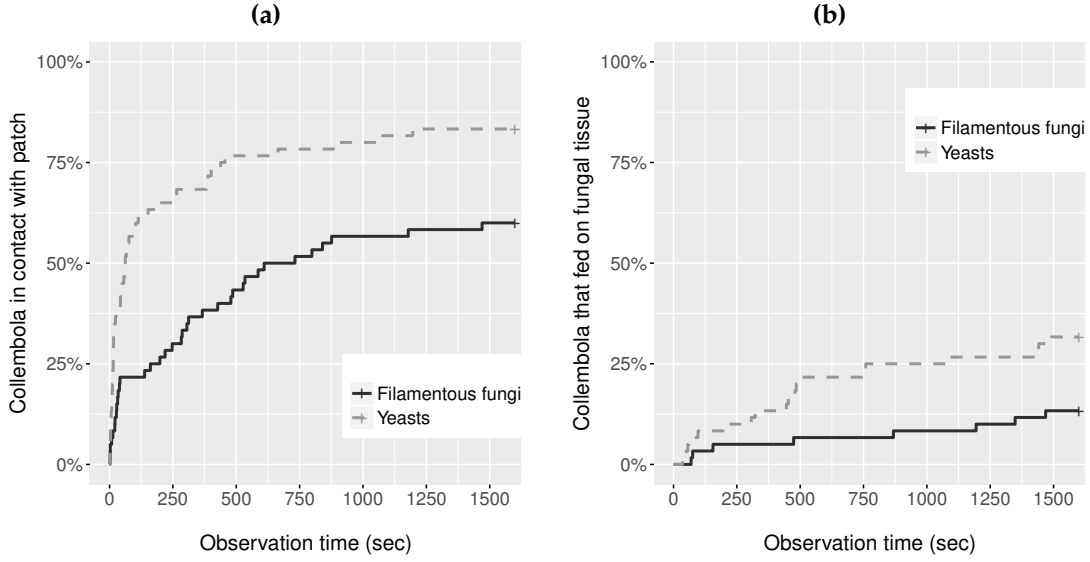

Figure 4.10: *Contact phase: Kaplan-Meier curves for* F. candida ***(a)*** *'latency to the first colony contact' (contact tendency) and* ***(b)*** *'latency to the first feeding event' (feeding tendency) in response to fungal life forms within an observation period of ~25 minutes.*

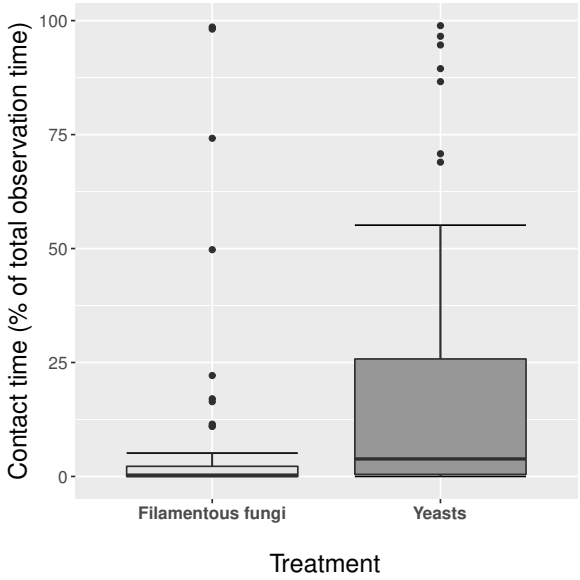

Figure 4.11: *Contact phase: 'contact time' of* F. candida *in response to fungal life forms.*

UNWOUNDED VS. WOUNDED COLONIES OF FILAMENTOUS FUNGI

Collembola contact behaviour in response to fungal tissue wounding was analysed with respect to filamentous fungi *A. nidulans*, *P. expansum*, and *T. harzianum*. No significant effects of wounding on behavioural variables were found (Table 4.14).

Table 4.14: *Contact phase: test statistic for 'latency to the first contact' (contact tendency), 'duration of the first contact' (departure tendency), 'latency to the first feeding event' (feeding tendency) (Cox regression), and 'contact time' (Wilcoxon test) in response to unwounded and wounded fungal colonies (pooled data). For Cox regression models $\tilde{\chi}^2$ values, and for Wilcoxon tests the Z-score is given. Effect size is represented by the hazard ratio ($\exp(coef)^{(*)}$) and Cohen's d, with 95% confidence interval.*

Behavioural variable	*N*	*Events*	*Df*	$\tilde{\chi}^2/Z$	*Effect size (CI)*	$P-value$
First contact	120	79	1	1.06	1.26	0.303
Duration first contact	79	77	1	0.06	1.06	0.810
First feeding	120	17	1	0.07	1.14	0.791
Contact time	120		1	-1.16	-0.12 (-0.48, 0.24)	0.248

Given effect sizes relate to the wounding treatment group. With respect to the latency to the first contact and feeding event a hazard ratio greater than 1 indicates higher contact and/or feeding tendency in the wounding group, and a value smaller than 1 lower contact and/or feeding tendency, respectively. Regarding the duration of the first visit a value smaller than 1 indicates higher tendency for remaining (i.e. lower departure tendency) in contact with wounded colonies.

4.4.4 EFFECT OF DIFFERENT FUNGAL DIETS ON COLLEMBOLA FITNESS

Reproduction (number of eggs) (Hurdle negative binomial regression, Wald test, $\tilde{\chi}^2$=160.44, df=8, p<0.001) and growth (change in body length) (Gamma regression, likelihood ratio test, $\tilde{\chi}^2$=195.72, df=7, p<0.001) of *F. candida* Collembola were significantly affected by the fungal diet and a strong correlation was found between these fitness parameters (r=0.71, 95% CI: 0.60-0.79, t=10.72, df=115, p<0.001). As many zeros were present in the egg data set a hurdle model was applied and revealed that the fungal diet had a significant effect on the number of eggs, but not on the zero count part ($\tilde{\chi}^2$=2.71, p=0.100), however, the parameter growth had a strong effect on zero egg counts (z=4.56, p<0.001). That means that the probability for laying no eggs was highest for those individuals that had low or no increase in growth, which is true for both control treatments, water agar and MEA, where only 37.5% and 64.7% of *F. candida* individuals, respectively, laid eggs, and also for the filamentous fungus *P. expansum*, where only 33.3% of individuals laid eggs (Figure 4.13a). In the presence of yeasts *C. terricola*, *M. pulcherrima*, and *T. dulcitum*, and filamentous fungi *A. nidulans* and *T. harzianum*, growth and oviposition was significantly increased compared to the water agar control (Figure 4.13, Table 4.15). Collembola performed best in the presence of the yeast *T. dulcitum*.

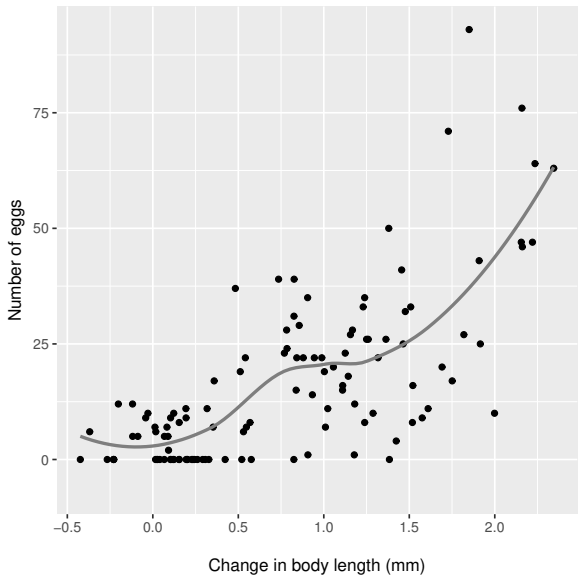

Figure 4.12: *Correlation between* F. candida *fitness parameters reproduction (number of eggs) and growth (change in body length) with lowess line (grey).*

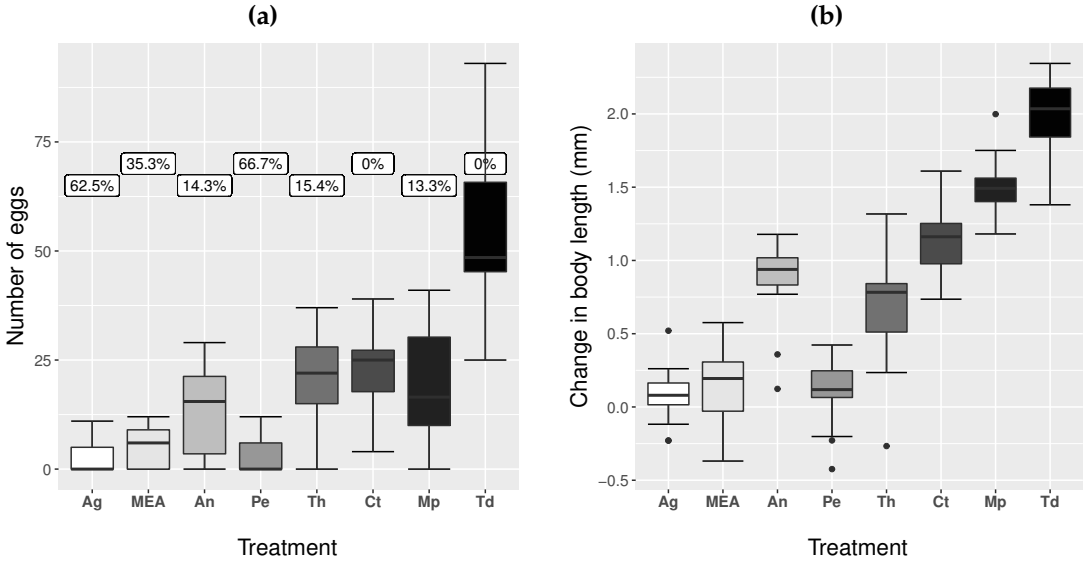

Figure 4.13: F. candida ***(a)*** *reproduction (number of eggs) and* ***(b)*** *growth (change in body length) in response to filamentous fungi* A. nidulans *(An),* T. harzianum *(Th), and* P. expansum *(Pe), yeasts* C. terricola *(Ct),* M. pulcherrima *(Mp), and* T. dulcitum *(Td), water agar controls (Ag) and MEA controls (MEA). Percent numbers represent the percentage of individuals that laid no eggs. Collembola were exposed to fungal diets for 12 days.*

Table 4.15: *Collembola fitness: test statistic for parameters 'reproduction' (number of eggs) and 'growth' (change in body length) in response to fungal diets. In case of the Gamma regression (growth) the t-value and in Hurdle negative binomial regression (reproduction) the z-value is given in the column 'Statistics'. Effect size is represented by the odds ratio (exp(coef)) with 95% confidence interval.*

Dietary treatment	*Fitness parameter*	n	*Statistics*	$\exp(coef)\ (CI)$	$P-value$	$^{(*)}$
Water agar control	Reproduction	16	8.11	6.59 (4.18, 10.39)	**<0.001**	
MEA control	Reproduction	17	0.66	1.12 (0.69, 2.11)	0.509	
A. nidulans	Reproduction	14	3.24	2.40 (1.41, 4.08)	**0.001**	▲
T. harzianum	Reproduction	13	4.69	3.54 (2.09, 6.02)	**<0.001**	▲
P. expansum	Reproduction	15	0.64	1.24 (0.64, 2.39)	0.525	
C. terricola	Reproduction	16	4.80	3.46 (2.08, 5.75)	**<0.001**	▲
M. pulcherrima	Reproduction	14	4.27	3.10 (1.85, 5.22)	**<0.001**	▲
T. dulcitum	Reproduction	12	8.02	8.24 (4.92, 13.81)	**<0.001**	▲
Water agar control	Growth	16	1.70	1.08 (0.99, 1.17)	0.092	
MEA control	Growth	17	1.21	1.08 (0.96, 1.21)	0.230	
A. nidulans	Growth	14	8.74	1.73 (1.53, 1.96)	**0.001**	▲
T. harzianum	Growth	13	6.84	1.55 (1.37, 1.76)	**<0.001**	▲
P. expansum	Growth	15	0.37	1.02 (0.91, 1.15)	0.716	
C. terricola	Growth	16	11.23	1.98 (1.76, 2.23)	**<0.001**	▲
M. pulcherrima	Growth	14	13.39	2.33 (2.06, 2.63)	**<0.001**	▲
T. dulcitum	Growth	12	15.53	2.78 (2.44, 3.16)	**<0.001**	▲

$^{(*)}$ Relative to the water agar control group ▲ a significant increase in the respective fitness parameter.

4.5 DISCUSSION

Several studies suggest a significant role of fungal volatiles in influencing the foraging behaviour of Collembola (Auclerc *et al.* 2010, Bengtsson *et al.* 1988; 1991; 1994, Hedlund *et al.* 1995, Sadaka-Laulan *et al.* 1998, Staaden *et al.* 2011). However, the relationship between the volatile-mediated foraging behaviour, the acceptance of a fungus as food by Collembola, and its fitness consequences for the animals is still unknown. Therefore, in the present study I tested whether Collembola use fungal-derived volatiles to differentiate between fungi of varying suitability by observing the foraging behaviour and fitness in response to different yeasts (*C. terricola*, *M. pulcherrima*, *T. dulcitum*) and filamentous fungi (*A. nidulans*, *T. harzianum*, *P. expansum*).

Overall, volatile-mediated foraging responses of Collembola were positively correlated with Collembola fitness measures, indicating that fungal volatiles are used as cues by Collembola to detect suitable and unsuitable fungi from a distance. This finding further strengthens the general assumption that the chemical signature of fungi plays an important role in affecting Collembola foraging and food selection (Böllmann *et al.* 2010, Hedlund *et al.* 1995, Rohlfs and Churchill 2011, Stötefeld *et al.* 2012) and suggests that the use of volatile and non-volatile fungal chemical cues as information is crucial for Collembola to optimize their fitness.

In line with expectations, a comparison of volatile-mediated movement patterns and contact behaviour of *F. candida* Collembola in response to fungal groups - yeasts and filamentous fungi (analysis of pooled data) - revealed that Collembola were more strongly attracted and arrested by yeasts. Moreover, yeasts were more accepted as food source than filamentous fungi when direct contact was allowed. However, these results should be considered with caution because behavioural responses to individual yeast and filamentous fungal species strongly differed, indicating fungal food-specific foraging in Collembola. When the yeast *C. terricola* and the filamentous fungus *P. expansum*, whose volatile bouquets were found to be the most attractive and most deterrent ones respectively, were excluded from the pooled data analysis, the effect of fungal groups on Collembola movement patterns became insignificant. Also when focussing on the number of feeding events in response to filamentous fungi, in contrast to *T. harzianum* and *P. expansum* for each of which only one feeding event was observed, *A. nidulans* was consumed by six out of 20 individuals and thus more accepted as food source. In the light of this variability in responses within fungal groups, the following discussion will mainly be limited to the effect of individual fungal species.

4.5.1 COLLEMBOLA RESPONSES TO FILAMENTOUS FUNGI

One of the most striking findings to emerge from behavioural observations and the fitness assay is that, among the other tested fungi, exclusively the volatile bouquet of the filamentous fungus *P. expansum* deterred the animals. In line with this Collembola rejected *P. expansum* as food source and had the lowest growth and reproduction when this fungus was offered as single diet. This strongly suggests that *P. expansum* characteristic volatile and/or non-volatile compounds function as deterrents and reduce the suitability of this fungus as food source. Among several terpenoid compounds that distinguish the volatile profile of *P. expansum* from those of the other tested fungi, the compound geosmin (trans-1,10-di-methyl-trans-9-decalol) was tested on affecting Collembola movement patterns and found to be, at least to some extent, responsible for the deterrent effect. This finding is in line with Stensmyr *et al.* (2012) who demonstrated that geosmin reduced attraction of fruit flies to vinegar. Another *Penicillium* species, which is also known to produce the terpenoid geosmin (Siddique 2012), was tested by Sadaka-Laulan *et al.* (1998) and demonstrated to be less attractive than other fungi. A comparable deterrent effect of other *P. expansum*-derived volatiles, styrene and 3-methylanisole, was also observed in pine weevils *Hylobius abietis* (Azeem *et al.* 2013), suggesting that other compounds could also be involved in affecting the foraging behaviour of Collembola. Preference tests also revealed that *P. expansum* was less attractive and accepted as food source compared to other fungi (Heděnec *et al.* 2013) which further supports the assumption that *P. expansum* is unsuited as food source for Collembola. The rejection of *P. expansum* as food source may also be related to the presence of mycotoxins, e.g. patulin and citrinin (Bennett *et al.* 2003), however, direct evidence for pathogenic effects of *P. expansum* characteristic mycotoxins on arthropods is still missing. In agreement with previous studies that attribute a significant role to fungal secondary metabolites as defence against fungivorous animals (e.g. Böllmann *et al.* 2010, Caballero Ortiz *et al.* 2013, Stötefeld *et al.* 2012), I suggest that the emission of deterrent volatiles, in particular terpenoid compounds such as geosmin, constitute an effective defence mechanism of *P. expansum*.

Observation of behavioural responses during the contact phase revealed that the filamentous fungus *T. harzianum*, similar to *P. expansum*, was the least accepted fungus. Surprisingly, despite the high complexity of its volatile profile and the emission of a range of terpenoid compounds, the volatile bouquet of *T. harzianum* did not evoke any response, implying that non-volatile metabolites and/or other factors, rather than volatiles, may have served as cues for Collembola to assess the suitability of this fungus. While acceptance was

low within the observation time of 25 minutes (contact behaviour), when *T. harzianum* was given as single diet for several days, Collembola growth and reproduction was positively affected. This finding indicates that the initial rejection turns into acceptance when Collembola are exposed to *T. harzianum* colonies for a longer time period and it can be assumed that *T. harzianum* is a suitable food source for Collembola. *Trichoderma* spp. form clumps of sticky spores within a mesh of hyphae which may impede the initial accessibility. In fact, Collembola were incidentally observed to be hindered in their movement on *T. harzianum* colonies and in some cases Collembola got stuck and were not able to break free. Both filamentous fungi - *T. harzianum* and *A. nidulans* - produce a range of toxins (Bräse *et al.* 2009, Rohlfs and Churchill 2011), are known as plant-, insect-, and fungal pathogens and therefore used as biocontrol agents against different pest species (Benítez *et al.* 2004, Morath *et al.* 2012, Omar A. Abdul-Wahid 2012). Despite the toxicity, *F. candida* Collembola increased growth and laid eggs when these fungi were given as single diets. This finding is in line with previous studies that have demonstrated that both fungi represent palatable food sources for *F. candida* (Klironomos and Kendrick 1996, Scheu and Simmerling 2004, Staaden *et al.* 2010). In contrast to *F. candida*, *Pseudosinella alba* rejected *T. harzianum* as food source and ceased their reproduction when this fungus was given as single diet (Ponge and Charpentié 1981). *F. candida* Collembola are known to have a storage detoxification system; waste products, toxins, and other harmful compounds are stored in the midgut cells of *F. candida* and excreted during moulting (Fountain and Hopkin 2005). This mechanism may enable *F. candida* Collembola to feed on toxin-producing fungi like *T. harzianum*. Different Collembola species, however, seem to differ in their detoxification efficiency as suggested by (Staaden *et al.* 2010), which may explain observed species-specific foraging responses (e.g. Chapter 2, Hedlund *et al.* (1995), Staaden *et al.* (2010)), feeding preferences for, and fitness consequences of fungal food sources (e.g. Larsen *et al.* 2008, Walsh and Bolger 1990).

4.5.2 COLLEMBOLA RESPONSES TO WOUND-ACTIVATED CHANGES IN FILAMENTOUS FUNGI

In the second chapter of this thesis I could show that the activation of oxylipin volatiles caused by fungal tissue wounding increased the acceptance of the saprotrophic fungus *C. globosum* by *F. candida* and the oxylipin 3-octanone is suggested to function as phagostimulant since this compound induced test-biting behaviour in two Collembola species

(*F. candida*, *S. curviseta*) (Chapter 2). Wounding of *P. expansum*, *T. harzianum*, and *A. nidulans* also mainly activated the emission of 3-octanone and other oxylipin volatiles, however, increased headspace concentrations of these compounds unexpectedly did not affect volatile-mediated searching patterns of *F. candida* nor acceptance of respective fungi as food source. Possibly, other compounds simultaneously emitted with volatile oxylipins might interfere with the otherwise feeding-stimulating effect of 3-octanone.

4.5.3 COLLEMBOLA RESPONSES TO UNICELLULAR YEASTS

Results of behavioural experiments indicate that the volatile bouquet of the yeast *C. terricola* had an arresting effect on Collembola, while the volatile bouquets of the yeasts *M. pulcherrima* and *T. dulcitum* attracted Collembola. However, attraction to these yeasts was only observed when Collembola were very close to the fungus (maximum 3 cm). This finding agrees with Auclerc *et al.* (2010) who have demonstrated that food-derived volatiles evoked *F. candida* Collembola to shift from random to directed movement only from a maximum distance of 2.5 cm. Blind or eye-reduced soil-dwelling (euedaphic, hemiedaphic) invertebrates, including *F. candida*, can be assumed to rely on the use of volatile cues to locate food from a distance. However, in the complex obstacle rich soil environment the transport of volatiles mainly happens via diffusion and may not be detectable over longer distances. Taking into account the composition of the volatile profiles of these yeasts, results indicate that the presence of the ubiquitous alcoholic compound 3-methyl-1-butanol within an otherwise volatile-poor background may be responsible or at least contribute to the attractiveness of the yeasts. As proposed by Rotheray *et al.* (2009), physiological characteristics may affect the accessibility and consumption of fungi. I suggest that the unicellular nature of yeasts and a less expansive hyphal growth of some filamentous fungi facilitate their consumption by Collembola, however, this hypothesis has to be tested in future experiments.

4.5.4 CONCLUSION

In conclusion, the present study clearly demonstrates that Collembola display fungal food-specific foraging. Volatile-mediated behavioural responses and feeding decisions (acceptance) of Collembola were well reflected in the fitness of the animals, indicating that the use

of volatile cues by Collembola is important for the detection of suitable and less suitable fungal food sources. Behavioural responses to the terpenoid compound geosmin strongly suggest that the emission of deterrent fungal volatiles represents an effective defence mechanism against fungivory. Since fungal volatile profiles and behavioural responses of Collembola are highly variable, it is not possible to make general assumptions on the effect of fungal groups (yeasts, filamentous fungi). Moreover, single volatile compound effects, in particular the phagostimulant effect of the wound-activated oxylipin 3-octanone, are supposed to depend on the composition of volatile profiles.

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APPENDIX

Table A.4.1: *Analytical response (manually integrated TIC peak area) to authentic geosmin diluted with paraffin at four concentrations (conc) (corresponding calibration plot: Figure A.2.1). The average TIC peak area of geosmin obtained from chromatograms of unwounded and wounded* P. expansum *colonies is 2312126 (log(TIC)=6.34).*

$Concentration$	$TIC\ peak\ area$	$log(Concentration)$	$log(TIC\ peak\ area)$
0.000001	67643	-6	4.83
0.00001	101382	-5	5.01
0.0001	842912	-4	5.93
0.001	5494647	-3	6.74

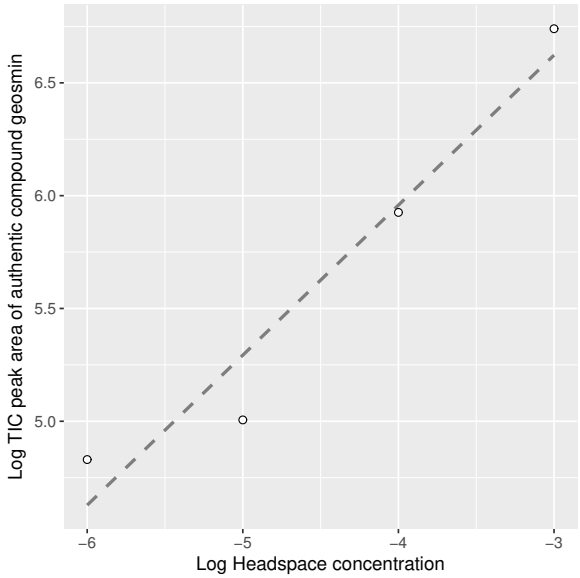

Figure A.4.1: *Logarithmic calibration plot with linear trendline (dotted, gray) representing analytical response (TIC peak area) as a function of known analyte concentration for the authentic volatile compound geosmin (adjusted R^2 = 0.9999, intercept = 132892, slope = 5378053261, p = 0.004). The average TIC peak area of geosmin obtained from chromatograms of unwounded and wounded* P. expansum *colonies is 2312126 (log(TIC)=6.34). Respective linear model estimates were used for quantification of the average geosmin headspace concentration in* P. expansum *colonies (formula: $x = (2312126 - 5378053261)/132892$; $log(x) = -3.61$). This calculation revealed an average geosmin headspace concentration of 25^{-4} (result of $10^{(log(x))}$) in* P. expansum *colonies.*

Table A.4.2: *Presence (black circle) and absence (white circle) of volatile compounds identified from headspace samples of* C. terricola, M. pulcherrima, T. dulcitum *yeast colonies, vegetative (VEG) and sporulating (SPO), unwounded and wounded (WO) colonies of filamentous fungi* A. nidulans *(genotypes: VeA1, VeA+),* T. harzianum, P. expansum *(genotypes: A, B), and fungal-free MEA medium controls. Presence was assumed if a compound was detected in two out of six replicates.*

		MEA (control)	*C.terricola*	*M.pulcherrima*	*T.dulcitum*	*A.nidulans* VeA1 VEG	*A.nidulans* VeA1 SPO	*A.nidulans* VeA1 WO VEG	*A.nidulans* VeA1 WO SPO	*A.nidulans* VeA+ VEG	*A.nidulans* VeA+ SPO	*A.nidulans* VeA+ WO VEG	*A.nidulans* VeA+ WO SPO	*P.expansum* A VEG	*P.expansum* A SPO	*P.expansum* A WO VEG	*P.expansum* A WO SPO	*P.expansum* B VEG	*P.expansum* B SPO	*P.expansum* B WO VEG	*P.expansum* B WO SPO	*T.harzianum* VEG	*T.harzianum* SPO	*T.harzianum* WO VEG	*T.harzianum* WO SPO					
Compound	CAS																									Identification *	RI_{Tc} [1]	RI_{LI} [2]	RI_{Ac} [3]	RI_{MF} [4]	Reference (RI_{L})
Acetates																														
1-octen-3-ol acetate	2442-10-6	○	○	○	○	○	○	●	●	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	1112	1112	-	-	Benzo et al. (2007)
3-octanol acetate	4864-61-3	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	1126	1127	1126	-	Andriamaharavo (2014)
Ethyl acetate	141-78-6	●	●	●	○	●	●	●	●	●	○	●	○	●	●	●	●	●	●	●	●	●	○	●	○	NISTms	626	612	-	-	Madruga & Mottram (1998)
Isoamyl acetate	123-92-2	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	887	878	-	-	Moio et al. (2000)
Isobutyl acetate	110-19-0	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	784	788	-	-	Fang & Qian (2005)
Alcohols																														
1-octen-3-ol	3391-86-4	○	○	○	○	●	○	●	●	●	○	●	●	●	○	●	●	○	○	●	●	○	●	●	●	NISTms	982	982	-	-	Moio et al. (2000)
1-propanol	71-23-8	●	●	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○	●	○	NISTms	588	595	-	-	Bylaite & Meyer (2006)
2-methyl-1-propanol	78-83-1	○	○	●	○	○	○	○	○	○	○	○	○	●	●	○	●	○	●	○	●	●	○	●	○	NISTms	645	654	643	-	Bylaite & Meyer (2006)
2-methyl-3-buten-2-ol	115-18-4	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	○	○	○	NISTms	635	620	631	-	Pino et al. (2003)
2-propanol	67-63-0	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	524	516	-	-	Xu et al. (2003)
3-methyl-3-buten-1-ol	763-32-6	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	743	734	743	-	Moio & Addeo (1998)
Ethanol	64-17-5	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	479	446	-	-	Peng (1992)
Isoamyl alcohol	123-51-3	○	●	●	●	●	●	●	●	●	○	●	○	●	●	●	●	●	●	●	●	●	○	●	○	NISTms	745	750	745	-	Komárek et al. (1998)
Phenylethyl alcohol	60-12-8	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	1140	1141	-	-	Rout et al. (2007)
Aldehydes																														
2-methylbutanal	96-17-3	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	668	664	-	-	Rychlik & Bosset (2001)
3-methylbutanal	590-86-3	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	662	664	-	-	Bylaite & Meyer (2006)
Hexanal	66-25-1	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	803	803	-	-	Wu & Cadwallader (2002)
Alkenes																														
1,3-octadiene	1002-33-1	○	○	○	○	○	○	●	●	○	○	●	●	○	○	●	●	○	○	●	●	○	○	●	●	NISTms	826	826	-	-	Methven et al. (2007)
1,3-trans,5-trans-octatriene	40087-62-5	○	○	○	○	○	○	●	●	○	○	●	●	○	○	●	●	○	○	●	●	○	○	○	●	NISTms	886	880	-	-	Xie et al. (2007)
2,4,6-octatriene	764-75-0	○	○	○	○	○	○	●	●	○	○	●	●	○	○	●	●	○	○	●	●	○	○	○	●	NISTms	937	925	-	-	Montanarella et al. (1986)
3,5,5-trimethyl-2-hexene	26456-76-8	○	○	○	○	○	○	●	●	○	○	●	●	○	○	●	●	○	○	●	●	○	○	○	○	NISTms	979	985	-	-	Duflos et al. (2005)

To be continued

Table A.4.2: *Continuation*

Compound	CAS	MEA (control)	*C.terricola*	*M.pulcherrima*	*T.dulcitum*	*A.nidulans* VeA1 VEG	*A.nidulans* VeA1 SPO	*A.nidulans* VeA1 WO VEG	*A.nidulans* VeA1 WO SPO	*A.nidulans* VeA+ VEG	*A.nidulans* VeA+ SPO	*A.nidulans* VeA+ WO VEG	*A.nidulans* VeA+ WO SPO	*P.expansum* A VEG	*P.expansum* A SPO	*P.expansum* A WO VEG	*P.expansum* A WO SPO	*P.expansum* B VEG	*P.expansum* B SPO	*P.expansum* B WO VEG	*P.expansum* B WO SPO	*T.harzianum* VEG	*T.harzianum* SPO	*T.harzianum* WO VEG	*T.harzianum* WO SPO	Identification *	RI_{Tc} [1]	RI_{Li} [2]	RI_{Ac} [3]	RI_{MF} [4]	Reference (RI_{Li})
Aromatic compounds																															
m-methylanisole	100-84-5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	○	○	○	NISTms	1026	-	-	-	
Heterocyclic compounds																															
2-methylfuran	534-22-5	●	●	●	●	●	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	608	605	-	-	Methven et al. (2007)
Furan	110-00-9	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	502	-	502	-	
Inorganic gases																															
CO_2	124-38-9	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	-	-	-	-	
N_2	7727-37-9	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	-	-	-	-	
Ketones																															
2-butanone	78-93-3	●	●	●	●	●	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	604	603	-	-	Madruge & Mottram (1998)
2,3-butanedione	431-03-8	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	NISTms	603	606	-	-	Mahajan et al. (2004)
3-octanone	106-68-3	○	○	○	○	●	○	●	●	○	○	●	○	●	○	●	●	○	○	●	●	●	○	●	●	NISTms	988	988	988	-	Javidnia et al. (2005)
5-methyl-3-heptanone	541-85-5	●	●	○	●	●	○	●	○	○	○	○	○	●	○	●	○	○	●	●	●	●	●	●	●	NISTms	943	939	-	-	Rostad & Pereira (1986)
Acetone	67-64-1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	506	503	-	-	Insausti et al. (2005)
Sesquiterpenes																															
(3E,6Z)-α-farnesene	502-61-4	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	MFms	1473	1475	-	1475	Ziegenbein et al. (2006)
(3Z,6E)-α-farnesene	26560-14-5	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	MFms	1483	1484	-	1484	Sabulal et al. (2007)
7-epi-α-selinene	123123-37-5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	MFms	1517	1517	-	1519	Maia et al. (2000)
cis-α-bergamotene	18252-46-5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1410	1410	-	1411	Cao et al. (2011)
Daucene	16661-00-0	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1378	1380	-	1380	Bader et al. (2003)
Germacrene D	23986-74-5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	MFms	1477	1477	-	1479	Rout et al. (2007)
Isocalamenene	68566-70-1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1531	1524	-	1527	Parker et al. (2007)
Isocyclobazzanene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	MFms	1323	-	-	1319	
Nardosina-9,11-diene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1483	-	-	1484	

To be continued

Table A.4.2: *Continuation*

Compound	CAS	MEA (control)	*C.terricola*	*M.pulcherrima*	*T.dulcitum*	*A.nidulans* VeA1 VEG	*A.nidulans* VeA1 SPO	*A.nidulans* VeA1 WO VEG	*A.nidulans* VeA1 WO SPO	*A.nidulans* VeA+ VEG	*A.nidulans* VeA+ SPO	*A.nidulans* VeA+ WO VEG	*A.nidulans* VeA+ WO SPO	*P.expansum* A VEG	*P.expansum* A SPO	*P.expansum* A WO VEG	*P.expansum* A WO SPO	*P.expansum* B VEG	*P.expansum* B SPO	*P.expansum* B WO VEG	*P.expansum* B WO SPO	*T.harzianum* VEG	*T.harzianum* SPO	*T.harzianum* WO VEG	*T.harzianum* WO SPO	Identification *	RI_{Tc} [1]	RI_{Li} [2]	RI_{Ac} [3]	RI_{MF} [4]	Reference (RI_{Li})
Sesquiterpenes (continued)																															
Pacifigorgia-1(6),10-diene	351222-70-3	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1426	1414	-	1414	Paul et al. (2001)
Rosifoliol		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	MFms	1598	1599	-	1599	Ruberto et al. (2002)
Rotundene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1457	1461	-	1461	Mahmout et al. (2002)
Selina-4(15),5-diene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1431	-	-	1433	
Striatene	83920-96-1	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	●	○	●	MFms	1461	1461	-	1458	Coutois et al. (2009)
Thujopsene	470-40-6	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1441	1428	-	1434	Isidorov et al. (1998)
Trans-β-bergamotene	15438-94-5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	MFms	1480	-	-	1480	
Verticos-7(13)-ene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	MFms	1357	-	-	1357	
α-alaskene	28400-12-6	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1520	1514	-	1512	Coutois et al. (2009)
α-duprezianene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1389	1388	-	1388	Adams et al. (2004)
β-bazzanene		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1515	-	-	1519	
β-cedrene	546-28-1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1427	1421	-	1430	Su et al. (2006)
β-elemene	515-13-9	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	MFms, NISTms	1395	1394	-	1394	Blagojevic et al. (2006)
β-funebrene	79120-98-2	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1423	1414	-	1418	Adams et al. (2004)
Unknown (1)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1294	-	-	-	
Unknown (2)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1307	-	-	-	
Unknown (3)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1315	-	-	-	
Unknown (4)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	○	●	○	○	○	○	○		1340	-	-	-	
Unknown (5)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1353	-	-	-	
Unknown (6)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	○	○	○	○	○	○	○		1371	-	-	-	
Unknown (7)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	●	○	●	○	○	○	○		1374	-	-	-	
Unknown (8)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1385	-	-	-	
Unknown (9)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	○	●	○	○	○	○	○		1389	-	-	-	
Unknown (10)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○		1428	-	-	-	
Unknown (11)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	○	○	○	○	○	○	○		1440	-	-	-	
Unknown (12)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	○	○	○	○	○	○	○		1444	-	-	-	
Unknown (13)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	○	○	○	○	○	○	○		1452	-	-	-	

To be continued

Table A.4.2: *Continuation*

Compound	CAS	MEA (control)	*C.terricola*	*M.pulcherrima*	*T.dulcitum*	*A.nidulans* VEG	*A.nidulans* SPO	*A.nidulans* VeA1 WO VEG	*A.nidulans* VeA1 WO SPO	*A.nidulans* VeA+ VEG	*A.nidulans* VeA+ SPO	*A.nidulans* VeA+ WO VEG	*A.nidulans* VeA+ WO SPO	*P.expansum* A VEG	*P.expansum* A SPO	*P.expansum* A WO VEG	*P.expansum* A WO SPO	*P.expansum* B VEG	*P.expansum* B SPO	*P.expansum* B WO VEG	*P.expansum* B WO SPO	*T.harzianum* VEG	*T.harzianum* SPO	*T.harzianum* WO VEG	*T.harzianum* WO SPO	Identification *	RI_{Tc} [1]	RI_{Li} [2]	RI_{Ac} [3]	RI_{MF} [4]	Reference (RI_{Li})
Sesquiterpenes (continued)																															
Unknown (14)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1492	-	-	-	Barley & Jacobs (2000)
Unknown (15)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	MFms	1503	-	-	-	Rout et al. (2007)
Unknown (16)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○		1515	-	-	-	
Unknown (17)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1529	-	-	-	
Unknown (18)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		1542	-	-	-	
Unknown (19)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		1553	-	-	-	
Sesquiterpenoids																															
(E)-nerolidol	7212-44-4	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	NISTms	1568	1569	-	1553	Sabulal et al. (2007)
Aromadendran-14-ol		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	MFms	1678	-	-	1679	
Terpenes																															
2-methyl-2-bornene	72540-93-3	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	NISTms	1015	1021	-	-	Dickschat et al. (2005)
2-methylenebornane	27538-47-2	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	NISTms	983	-	-	-	
2methylisoborneol	2371-42-8	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	NISTms	1184	1182	1184	-	Mahmout & Buettner (2017)
Geosmin	19700-21-1	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○	MFms, NISTms	1413	1428	1420	1392	Selli et al. (2006)
Limonene	138-86-3	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	NISTms	1030	1030	-	-	Javidnia et al. (2003)
Unknown (20)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	○	○	○	○		1238	-	-	-	
Terpenoids																															
Dihydroedulan I	63335-66-0	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	○	○	○	NISTms	1299	1300	-	-	Zhao et al. (2005)
Unknown compounds																															
Unknown (21)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1326	-	-	-	
Unknown (22)		○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	○	○	○	○	○	○	○	○		1331	-	-	-	
Unknown (23)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1336	-	-	-	
Unknown (24)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1343	-	-	-	
Unknown (25)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●		1371	-	-	-	

To be continued

Table A.4.2: *Continuation*

Compound	CAS	MEA (control)	*C.terricola*	*M.pulcherrima*	*T.dulcitum*	*A.nidulans* VeA1 VEG	*A.nidulans* VeA1 SPO	*A.nidulans* VeA1 WO VEG	*A.nidulans* VeA1 WO SPO	*A.nidulans* VeA+ VEG	*A.nidulans* VeA+ SPO	*A.nidulans* VeA+ WO VEG	*A.nidulans* VeA+ WO SPO	*P.expansum* A VEG	*P.expansum* A SPO	*P.expansum* A WO VEG	*P.expansum* A WO SPO	*P.expansum* B VEG	*P.expansum* B SPO	*P.expansum* B WO VEG	*P.expansum* B WO SPO	*T.harzianum* VEG	*T.harzianum* SPO	*T.harzianum* WO VEG	*T.harzianum* WO SPO	Identification *	RI_{Tc} [1]	RI_{Li} [2]	RI_{Ac} [3]	RI_{MF} [4]	Reference (RI_{Li})
Unknown compounds (continued)																															
Unknown (26)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		1397	-	-	-	
Unknown (27)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	●	●	●	●	○	○	○	○		1556	-	-	-	
Unknown (28)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		1700	-	-	-	
Unknown (29)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		1725	-	-	-	
Unknown (30)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		1753	-	-	-	
Unknown (31)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		1767	-	-	-	
Unknown (32)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (33)		○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (34)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (35)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (36)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		-	-	-	-	
Unknown (37)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (38)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (39)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		-	-	-	-	
Unknown (40)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		-	-	-	-	
Unknown (41)		○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	○	●	○	○	○	○		-	-	-	-	
Unknown (42)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		-	-	-	-	
Unknown (43)		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●		-	-	-	-	

[1] Van den Dool and Kratz retention indices of target compounds; [2] Van den Dool and Kratz retention indices from literature; [3] Van den Dool and Kratz retention indices of authentic standards; [4] Van den Dool and Kratz retention indices from MassFinder 4 Terpenoids Library

* Identification by mass spectrum of NIST library with minimum match of 800 (NISTms), and/or Mass Finder Terpenoids Library (MFms)

Table A.4.3: *Wilcoxon test statistic - Effect of fungal tissue wounding on quantities (manually integrated peak areas) of volatile compounds detected in headspace samples of vegetative and sporulating colonies of filamentous fungi* A. nidulans *(two genotypes),* P. expansum *(two genotypes), and* T. harzianum. *Naturally, wound-activated compounds that were solely detected in wounded colonies were not tested and thus are not mentioned in this table.*

Compound	Z	$P-value$
***A. nidulans*, VeA1, vegetative**		
3-methyl-1-butanol	-0.16	0.937
1-octen-3-ol	-2.91	**0.002**
3-octanone	-2.88	**0.002**
***A. nidulans*, VeA1, sporulating**		
3-methyl-1-butanol	-0.26	0.870
***A. nidulans*, VeA+, vegetative**		
3-methyl-1-butanol	-0.80	0.455
1-octen-3-ol	-2.88	**0.002**
***A. nidulans*, VeA+, sporulating**		
(no compounds detected)		
***P. expansum*, A, vegetative**		
3-methyl-1-butanol	-0.80	0.485
1-octen-3-ol	-2.89	**0.002**
3-octanone	-2.72	**0.004**
(3Z,6E)-α-farnesene	-0.16	0.937
Rosifoliol	1.12	0.310
Striatene	-0.16	0.937
β-elemene	0.16	0.937
(E)-Nerolidol	1.66	0.113
2-methylenebornane	0.32	0.818
2-methyl-2-bornene	1.12	0.310
2-methyisoborneol	0.96	0.394
Geosmin	0.16	0.937
Unknown (4)	0.80	0.485
Unknown (6)	-0.26	0.870
Unknown (7)	1.44	0.169
Unknown (9)	0.32	0.818
Unknown (10)	0.96	0.394
Unknown (11)	0.32	0.814
Unknown (12)	1.44	0.180
Unknown (13)	0.48	0.700
Unknown (15)	0.80	0.485
Unknown (16)	-0.80	0.485
Unknown (20)	0	1
Unknown (22)	0.16	0.937
Unknown (33)	0.16	0.939

To be continued

Table A.4.3 *Continuation*

***P. expansum*, A, sporulating**		
2-methyl-1-propanol	0.80	0.485
3-methyl-1-butanol	-0.64	0.589
(3Z,6E)-α-farnesene	0.54	0.697
Rosifoliol	-0.32	0.777
Striatene	0.90	0.407
β-elemene	-0.16	0.937
(E)-Nerolidol	1.33	0.221
2-methylenebornane	-0.64	0.589
2-methyl-2-bornene	0.32	0.818
2-methyisoborneol	0	1
Geosmin	0.16	0.937
Unknown (4)	0.54	0.697
Unknown (6)	0	1
Unknown (7)	-1.20	0.255
Unknown (9)	0.60	0.590
Unknown (10)	1.13	0.307
Unknown (11)	-0.89	0.394
Unknown (12)	1.47	0.147
Unknown (13)	-0.32	0.818
Unknown (15)	0.16	0.937
Unknown (16)	-1.12	0.310
Unknown (20)	-0.64	0.589
Unknown (27)	0.16	0.937
Unknown (29)	-0.96	0.394
Unknown (32)	0	0
Unknown (33)	0.48	0.699
Unknown (34)	-0.80	0.485
Unknown (35)	0.16	0.937
Unknown (37)	0.64	0.589
Unknown (38)	-0.48	0.699
Unknown (41)	0	1
***P. expansum*, B, vegetative**		
3-methyl-1-butanol	-1.12	0.310
(3Z,6E)-α-farnesene	-0.48	0.699
Rosifoliol	1.92	0.065
Striatene	-0.32	0.818
β-elemene	-0.16	0.937
(E)-Nerolidol	0.24	0.859
2-methylenebornane	-0.43	0.708
2-methyl-2-bornene	-0.16	0.937
2-methylanisole	-1.60	0.132
2-methyisoborneol	-1.28	0.240

To be continued

Table A.4.3 *Continuation*

Geosmin	-1.44	0.180
Unknown (4)	0	1
Unknown (9)	0	1
Unknown (10)	1.76	0.093
Unknown (15)	-1.76	0.093
Unknown (16)	-1.44	0.180
Unknown (20)	-1.28	0.240
Unknown (27)	-0.50	0.697
Unknown (33)	0.16	0.939
***P. expansum*, B, sporulating**		
2-methyl-1-propanol	0.48	0.699
2-methyl-3-buten-2-ol	-1.12	0.310
3-methyl-1-butanol	-1.28	0.240
(3Z,6E)-α-farnesene	-0.48	0.699
Rosifoliol	-0.08	0.981
Striatene	-1.49	0.167
β-elemene	-0.16	0.937
(E)-Nerolidol	-0.33	0.805
2-methylenebornane	-2.24	**0.026**
2-methyl-2-bornene	-0.48	0.699
2-methylanisole	-0.57	0.606
2-methyisoborneol	-1.44	0.180
Geosmin	0.32	0.818
Dihydroeludan I	-1.28	0.240
Unknown (7)	-0.32	0.818
Unknown (10)	0.32	0.818
Unknown (15)	-0.32	0.777
Unknown (16)	-0.96	0.394
Unknown (20)	-0.16	0.937
Unknown (27)	-0.48	0.699
Unknown (29)	-1.28	0.240
Unknown (30)	-1.28	0.240
Unknown (32)	-0.80	0.485
Unknown (33)	-1.12	0.310
Unknown (34)	0.08	0.981
Unknown (35)	-0.96	0.394
Unknown (37)	0.43	0.708
Unknown (38)	-0.64	0.589
Unknown (41)	-0.64	0.589
***T. harzianum*, vegetative**		
2-methyl-1-propanol	-0.64	0.589
3-methyl-1-butanol	-0.18	0.924
3-octanone	-1.99	0.058

To be continued

Table A.4.3 *Continuation*

Daucene	0	1
α-duprezianene	0.32	0.818
cis-α-bergamotene	-0.32	0.818
β-funebrene	0.32	0.818
Pacifigorgia-1(6),10-diene	0	1
β-cedrene	-0.16	0.937
Selina-4(15),5-diene	0.32	0.818
Thujopsene	0.64	0.589
Rotundene	0.80	0.485
trans-β-bergamotene	-2.24	**0.026**
Nardosina-9,11-diene	-0.16	0.937
β-bazzanene	0.48	0.699
α-alaskene	0.16	0.937
Isocalamenene	-0.16	0.937
Aromadendran-14-ol	-0.32	0.818
Unknown (14)	0.32	0.818
Unknown (15)	0.16	0.937
Unknown (18)	-0.16	0.937
Unknown (19)	-0.32	0.777
Unknown (26)	0.48	0.699
Unknown (28)	-0.64	0.589
Unknown (31)	0.64	0.589
Unknown (36)	0.64	0.554
Unknown (39)	0.80	0.485
Unknown (40)	-0.16	0.937
Unknown (42)	0.80	0.485
Unknown (43)	0.96	0.394
***T. harzianum*, sporulating**		
1-octen-3-ol	-2.93	**0.002**
(3Z,6E)-α-farnesene	-0.48	0.699
7-epi-α-selinene	-0.80	0.485
Daucene	-0.64	0.589
Germacrene D	-0.80	0.485
cis-α-bergamotene	-1.60	0.123
β-funebrene	-2.08	**0.041**
Pacifigorgia-1(6),10-diene	1.60	0.123
β-cedrene	-1.92	0.065
Selina-4(15),5-diene	-1.12	0.310
Striatene	-0.64	0.589
Thujopsene	1.60	0.123
Rotundene	1.60	0.123
Nardosina-9,11-diene	-2.40	**0.015**
Verticos-7(13)-ene	-0.64	0.589

To be continued

Table A.4.3 *Continuation*

α-alaskene	-1.76	0.093
α-duprezianene	-0.48	0.699
β-bazzanene	-0.50	0.697
Isocalamenene	-1.29	0.234
Isocyclobazzanene	-1.12	0.310
Aromadendran-14-ol	-1.28	0.240
Unknown (1)	-1.92	0.065
Unknown (2)	-1.28	0.240
Unknown (3)	-1.60	0.132
Unknown (5)	-0.64	0.589
Unknown (8)	-1.44	0.180
Unknown (14)	-1.12	0.310
Unknown (15)	-0.80	0.485
Unknown (17)	0.08	1
Unknown (18)	-1.76	0.093
Unknown (19)	-1.12	0.310
Unknown (21)	-0.96	0.394
Unknown (23)	-1.44	0.180
Unknown (24)	-0.16	0.937
Unknown (25)	-0.64	0.589
Unknown (26)	-2.08	**0.041**
Unknown (28)	-1.12	0.310
Unknown (31)	-1.60	0.123
Unknown (36)	0.41	0.706
Unknown (39)	0.16	0.937
Unknown (40)	0	1
Unknown (42)	-0.32	0.814
Unknown (43)	0	1

Table A.4.4: *On-patch contact behaviour raw data on the 'duration of the first feeding event' (First F dur), 'total feeding time' (Total feeding), and 'patch contact frequency prior feeding' (Contacts prior F).*

Observation number	*Treatment*	*First F dur (sec)*	*Time : feeding (%)*	*Contacts prior F (freq)*
1	*C. terricola*		0	
2	*C. terricola*		0	
3	*C. terricola*	41.792	55.75	0.0256
4	*C. terricola*		0	
5	*C. terricola*		0	
6	*C. terricola*	28.103	28.49	0.0199
7	*C. terricola*		0	
8	*C. terricola*		0	
9	*C. terricola*	58.558	3.90	0.0319
10	*C. terricola*		0	
11	*C. terricola*		0	
12	*C. terricola*		0	
13	*C. terricola*		0	
14	*C. terricola*		0	
15	*C. terricola*		0	
16	*C. terricola*		0	
17	*C. terricola*		0	
18	*C. terricola*		0	
19	*C. terricola*		0	
20	*C. terricola*	13.191	24.66	0.0177
1	*M. pulcherrima*		0	
2	*M. pulcherrima*	104.468	6.96	0.0465
3	*M. pulcherrima*	571.568	49.61	0.0022
4	*M. pulcherrima*		0	
5	*M. pulcherrima*		0	
6	*M. pulcherrima*	33.886	2.26	0.0330
7	*M. pulcherrima*		0	
8	*M. pulcherrima*		0	
9	*M. pulcherrima*		0	
10	*M. pulcherrima*		0	
11	*M. pulcherrima*	58.086	3.88	0.0292
12	*M. pulcherrima*		0	
13	*M. pulcherrima*	59.852	4.04	0.0040
14	*M. pulcherrima*		0	
15	*M. pulcherrima*	22.315	49.26	0.0065
16	*M. pulcherrima*		0	
17	*M. pulcherrima*		0	
18	*M. pulcherrima*		0	
19	*M. pulcherrima*		0	
20	*M. pulcherrima*		0	
1	*T. dulcitum*		0	
2	*T. dulcitum*	38.966	2.60	0.0042
3	*T. dulcitum*		0	
4	*T. dulcitum*	263.18	21.87	0.0037
5	*T. dulcitum*	247.042	16.47	0.0060
6	*T. dulcitum*	107.081	69.95	0.0207
7	*T. dulcitum*	61.272	67.24	0.0281
8	*T. dulcitum*	9.33	0.62	0.0034
9	*T. dulcitum*	149.01	46.48	0.0162
10	*T. dulcitum*		0	
11	*T. dulcitum*		0	
12	*T. dulcitum*		0	
13	*T. dulcitum*		0	
14	*T. dulcitum*		0	
15	*T. dulcitum*	58.347	3.89	0.0044
16	*T. dulcitum*		0	
17	*T. dulcitum*		0	
18	*T. dulcitum*		0	
19	*T. dulcitum*	80.605	28.13	0.0041
20	*T. dulcitum*		0	

To be continued

Table A.4.4 *Continuation*

Observation number	*Treatment*	*First F dur (sec)*	*Total feeding (%)*	*Contacts prior F (freq)*
1	*A. nidulans*		0	
2	*A. nidulans*		0	
3	*A. nidulans*	151.666	10.11	0.0334
4	*A. nidulans*		0	
5	*A. nidulans*	1.11	1.50	0.0530
6	*A. nidulans*	67.443	52.97	0.0337
7	*A. nidulans*		0	
8	*A. nidulans*	31.129	2.08	0.0109
9	*A. nidulans*	631.979	42.13	0.0104
10	*A. nidulans*	1391.867	95.25	0.0144
11	*A. nidulans*		0	
12	*A. nidulans*		0	
13	*A. nidulans*		0	
14	*A. nidulans*		0	
15	*A. nidulans*		0	
16	*A. nidulans*		0	
17	*A. nidulans*		0	
18	*A. nidulans*		0	
19	*A. nidulans*		0	
20	*A. nidulans*		0	
1	*P. expansum*		0	
2	*P. expansum*		0	
3	*P. expansum*		0	
4	*P. expansum*		0	
5	*P. expansum*		0	
6	*P. expansum*	13.716	1.62	0.0257
7	*P. expansum*		0	
8	*P. expansum*		0	
9	*P. expansum*		0	
10	*P. expansum*		0	
11	*P. expansum*		0	
12	*P. expansum*		0	
13	*P. expansum*		0	
14	*P. expansum*		0	
15	*P. expansum*		0	
16	*P. expansum*		0	
17	*P. expansum*		0	
18	*P. expansum*		0	
19	*P. expansum*		0	
20	*P. expansum*		0	
1	*T. harzianum*		0	
2	*T. harzianum*		0	
3	*T. harzianum*		0	
4	*T. harzianum*		0	
5	*T. harzianum*		0	
6	*T. harzianum*		0	
7	*T. harzianum*		0	
8	*T. harzianum*		0	
9	*T. harzianum*	20.486	1.37	0.0059
10	*T. harzianum*		0	
11	*T. harzianum*		0	
12	*T. harzianum*		0	
13	*T. harzianum*		0	
14	*T. harzianum*		0	
15	*T. harzianum*		0	
16	*T. harzianum*		0	
17	*T. harzianum*		0	
18	*T. harzianum*		0	
19	*T. harzianum*		0	
20	*T. harzianum*		0	

To be continued

Table A.4.4 *Continuation*

Observation number	*Treatment*	*First F dur (sec)*	*Total feeding (%)*	*Contacts prior F (freq)*
1	*A. nidulans* wounded	92.511	18.47	0.0021
2	*A. nidulans* wounded	55.246	4.86	0.0434
3	*A. nidulans* wounded		0	
4	*A. nidulans* wounded		0	
5	*A. nidulans* wounded		0	
6	*A. nidulans* wounded	71.950	7.24	0.0073
7	*A. nidulans* wounded	11.074	4.24	0.0145
8	*A. nidulans* wounded		0	
9	*A. nidulans* wounded		0	
10	*A. nidulans* wounded	17.665	2.83	0.1669
11	*A. nidulans* wounded		0	
12	*A. nidulans* wounded		0	
13	*A. nidulans* wounded		0	
14	*A. nidulans* wounded		0	
15	*A. nidulans* wounded	263.512	28.57	0.0147
16	*A. nidulans* wounded		0	
17	*A. nidulans* wounded		0	
18	*A. nidulans* wounded		0	
19	*A. nidulans* wounded		0	
20	*A. nidulans* wounded	12.975	0.87	0.0068
1	*P. expansum* wounded		0	
2	*P. expansum* wounded		0	
3	*P. expansum* wounded		0	
4	*P. expansum* wounded		0	
5	*P. expansum* wounded		0	
6	*P. expansum* wounded		0	
7	*P. expansum* wounded		0	
8	*P. expansum* wounded		0	
9	*P. expansum* wounded		0	
10	*P. expansum* wounded		0	
11	*P. expansum* wounded		0	
12	*P. expansum* wounded		0	
13	*P. expansum* wounded		0	
14	*P. expansum* wounded		0	
15	*P. expansum* wounded		0	
16	*P. expansum* wounded		0	
17	*P. expansum* wounded		0	
18	*P. expansum* wounded		0	
19	*P. expansum* wounded		0	
20	*P. expansum* wounded	12.982	0.87	0.0066
1	*T. harzianum* wounded		0	
2	*T. harzianum* wounded		0	
3	*T. harzianum* wounded		0	
4	*T. harzianum* wounded		0	
5	*T. harzianum* wounded	140.757	10.68	0.0109
6	*T. harzianum* wounded		0	
7	*T. harzianum* wounded		0	
8	*T. harzianum* wounded		0	
9	*T. harzianum* wounded		0	
10	*T. harzianum* wounded		0	
11	*T. harzianum* wounded		0	
12	*T. harzianum* wounded		0	
13	*T. harzianum* wounded		0	
14	*T. harzianum* wounded		0	
15	*T. harzianum* wounded		0	
16	*T. harzianum* wounded		0	
17	*T. harzianum* wounded		0	
18	*T. harzianum* wounded		0	
19	*T. harzianum* wounded		0	
20	*T. harzianum* wounded		0	

To be continued

Table A.4.4 *Continuation*

Observation number	*Treatment*	*First F dur (sec)*	*Total feeding (%)*	*Contacts prior F (freq)*
1	MEA control	380.705	25.38	0.0210
2	MEA control		0	
3	MEA control	135.906	9.06	0.0055
4	MEA control		0	
5	MEA control	167.397	25.10	0.0239
6	MEA control		0	
7	MEA control		0	
8	MEA control		0	
9	MEA control		0	
10	MEA control		0	
11	MEA control		0	
12	MEA control		0	
13	MEA control		0	
14	MEA control	356.408	23.76	0.0028
15	MEA control		0	
16	MEA control		0	
17	MEA control		0	
18	MEA control		0	
19	MEA control		0	
20	MEA control		0	

CHAPTER 5

FINAL DISCUSSION

In the light of their ubiquity, high diversity, abundance, and occurrence as primary colonisers, saprotrophic fungi are considered as major decomposers and key regulators of nutrient cycling in terrestrial ecosystems (Baldrian and Valášková 2008, Hättenschwiler *et al.* 2005, Kjoller and Struwe 1982, Watkinson *et al.* 2006). Increasing research on functional and ecological consequences of decomposer fungus-invertebrate interactions revealed that the interplay of fungi and soil animals plays a major role in shaping decomposition rates and related ecosystem processes. Primary colonisation and pre-decomposition of litter by fungi has been shown to enable or facilitate secondary colonisation by soil invertebrate decomposers and consequently affects the structure of soil faunal communities and population sizes (e.g. A'Bear *et al.* 2014a, Klironomos and Kendrick 1995, Men'ko *et al.* 2006, Richter 1979, Soma and Saito 1983, Wood *et al.* 2012, Zimmer and Topp 1997; 2000). Vice versa a wealth of studies has demonstrated that invertebrate fungal grazers directly affect the composition and activity of soil fungal communities by influencing enzyme activity, suppressing or encouraging fungal growth and altering amounts of competition via selective feeding and dispersal of fungal propagules and thus indirectly affect rates of decomposition and nutrient release (A'Bear *et al.* 2014a;b, Crowther *et al.* 2012; 2011a;b;c, David 2014, Hanlon and Anderson 1979, Jacobsen *et al.* 2017). Although the majority of soil invertebrates are considered as generalists that cover their nutritional requirements by feeding on a broad range of food sources, food-preference tests, stable isotope and gut content analysis revealed that fungi play a significant role in the diet of springtails, oribatid mites, annelids, isopods, diplopods, and molluscs (e.g. Anderson and Healey 1972, Berg *et al.* 2004, Kayang *et al.* 1996, Maraun *et al.* 2003, Pollierer *et al.* 2007, Ruf *et al.* 2006, Soma and Saito 1983, Zimmer 2002). Besides serving as food source for fungivorous invertebrates and acting as niche constructors for secondary colonisers, due to their diverse lifestyles fungi further directly and indirectly influence the performance of other soil organisms by serving as host, acting as pathogens and predators, and mediating multitrophic interactions (Chapter 1). A grand challenge in terrestrial ecology is to reveal the importance of ecological interactions and their direct and

indirect effects on ecosystem processes. With focus on the role of fungal-derived volatiles in mediating fungus-arthropod interactions, the present PhD project represents a further step towards a better understanding of the underlying mechanisms that shape the character of fungus-arthropod interactions.

5.1 THE ROLE OF FUNGAL VOLATILE OXYLIPINS IN MODULATING THE FORAGING BEHAVIOUR OF FUNGIVOROUS ARTHROPODS

The constitutive production and wounding-related activation/increase of oxylipin compounds is a conserved mechanism in plants and higher fungi and oxylipins are well known to fulfil multiple functions as growth regulators and, most notably, as communication signals and infochemicals in both aboveground plant-insect (e.g. Bruce *et al.* 2005, Heil 2014) and belowground fungus-invertebrate interactions (Holighaus and Rohlfs 2018, Tsitsigiannis and Keller 2007)(Chapter 1). Contrary to my expectations, the results of behavioural observations only partly confirm the hypothesis that fungal oxylipin volatiles function as food-finding cues for Collembola and isopods and that tissue wounding-related changes in fungal volatile profiles are used by these soil animals to make adaptive foraging and feeding decisions.

Independent of the wounding treatment, volatile emissions derived from fungal colonies attracted isopods and the mere presence of the common ubiquitous oxylipin volatile 3-octanone arrested them in close proximity to the volatile source. On the one hand this finding indicates that *O. asellus* isopods may not be able to perceive wounding-related changes in oxylipin emissions, and on the other hand suggests that 3-octanone functions as cue, most likely along with other fungal volatiles, that may help isopods to locate favourable microhabitats and feeding sites characterised by high microbial activity (Chapter 3). This supports (Zimmer *et al.* 1996) who observed attraction of *P. scaber* isopods to microbe-colonised litter. While studies by Zidar *et al.* (2003) and Gunnarsson (1987) suggest that food selection by *O. asellus* isopods requires physical contact to the fungal food source, the results of the present study for the first time demonstrate that these animals indeed use fungal-derived volatiles as infochemicals during foraging. Whereas the effect of fungal tissue wounding on the behaviour of isopods was only tested with respect to one fungal species (*C. globosum*) and restricted to the searching phase of food selection without physical contact, responses of

Collembola were observed in the presence of different fungal species and in more detail by additionally investigating the behaviour with direct contact to fungi to find out whether wounding-related increases in oxylipin emissions increase the acceptance fungi as food source. Similar to isopods, volatile-mediated movement patterns of hemi- and epedaphic Collembola *F. candida, S. curviseta, H. nitidus* were not affected by the wounding treatment. Since GC-MS analyses revealed significant differences in wounding-specific volatile profiles between the tested fungal species, *A. nidulans, P. expansum, T. harzianum* (Chapter 4), and *C. globosum* (Chapter 2), I assume that Collembola are generally not able to use wound-activated increases in oxylipin emissions to accelerate and optimise the food-finding process (Chapter 2, 4).

On the contrary, oxylipins, 3-octanone in particular, become relevant when Collembola get in contact with fungal colonies. Although responses of the three Collembola species to unwounded and wounded *C. globosum* colonies differed, altogether the results point to higher acceptance of wounded colonies as food source. Moreover, 3-octanone elicited test-biting behaviour in *F. candida* and *S. curviseta* suggesting that this common mushroom volatile functions as phagostimulant for Collembola. Test-biting behaviour has never been observed before in Collembola but frequently in herbivorous insects (e.g. Chapman and Bernays 1989, Schoonhoven *et al.* 2005). In the light of the finding that the wound-activated increase of 3-octanone in *A. nidulans, P. expansum, T. harzianum* did not affect the acceptance of these fungi as food source by *F. candida*, it can be assumed that the volatile background, i.e. the presence of other presumably deterrent volatile and/or non-volatile compounds, diminishes or erases the phagostimulant effect of 3-octanone.

5.2 COLLEMBOLA DISTINGUISH BETWEEN SUITABLE AND LESS SUITABLE FUNGAL FOOD SOURCES BY MEANS OF VOLATILES

Soil fungal communities not only comprise filamentous fungi but also a significant number of unicellular yeasts (Esteve-Zarzoso *et al.* 1999). While spores of filamentous fungi are primarily dispersed by wind, yeasts rely on animals that vector cells to new substrates; furthermore, due to their unicellular growth and nutritious value yeast present an easily consumable highly suitable food source (Blackwell 2017). Therefore, mutualistic relationships between yeasts and soil invertebrates are not seldom (Blackwell 2017) and yeast-derived

volatiles have frequently been shown to play an important role in mediating contact between the mutualist partners by acting as host-, food-, or habitat-finding cues (Chapter 1). In contrast, a range of filamentous fungi produces harmful and/or deterrent secondary metabolites that are supposed to have been evolved to defend against fungivory (Brandt *et al.* 2017, Kempken and Rohlfs 2010, Rohlfs *et al.* 2007, Sherratt *et al.* 2005, Stötefeld *et al.* 2012). In the light of these differences, I hypothesised that yeasts and filamentous fungi differ in their volatile emissions and that Collembola use characteristics in volatile profiles to differentiate between suitable and less suitable fungi.

Overall, the results confirm this hypothesis. Volatile-mediated responses to yeasts (*C. terricola*, *M. pulcherrima*, *T. dulcitum*) and filamentous fungi (*A. nidulans*, *P. expansum*, *T. harzianum*) were largely reflected in the acceptance and rejection of the tested fungi as food source and finally in the growth (changes in body size) and reproduction (number of eggs) when these fungi where given as single diet for 12 days, indicating that *F. candida* Collembola are indeed able to differentiate between suitable and less suitable fungi from a short distance (~3 cm) by means of volatile cues (Chapter 4). Responses to yeasts, however, were more unambiguous than those observed in the presence of the filamentous fungi. Contrasting effects may be explained by differences in fungal volatile profiles. More specifically, taking into account the results of the volatile profiling, the higher attractivity and/or acceptance of the yeasts and the filamentous fungus *A. nidulans* is likely due to low quantities of volatile emissions, the presence of the short-chain alcohol 3-methyl-1-butanol, and the absence of terpenoid compounds. The morphological properties of the tested fungi could be another factor possibly influencing the acceptance of fungal colonies as food source. While the tested yeasts grow unicellular, the filamentous fungi form a dense mesh of hyphae and spores which may impede Collembola from accessing and feeding on respective fungal colonies. Furthermore, the formation of sticky clumps of conidia characteristic for *Trichoderma* spp. may also play a role in influencing the acceptance as food source. This aspect needs to be tested in future studies.

Interestingly, exclusively the volatile bouquet of the filamentous fungus *P. expansum* had a deterrent effect, Collembola rejected this fungus as food source, indicated by an extremely low feeding activity, reproduction, and insignificant increase in growth. The observed deterrent effect of the *P. expansum* characteristic compound geosmin (trans-1,10-di-methyl-trans-9-decalol) strongly suggests that this compound, likely along with other deterrent and/or toxic volatile and/or non-volatile compounds, functions as chemical defence against fungivory and it can be assumed that Collembola are able to use respective compounds to de-

tect and avoid contact with harmful and unsuitable fungi. This assumption is strengthened by previous studies that also point out deterrent effects of geosmin (Stensmyr *et al.* 2012) and other *P. expansum* characteristic volatiles (Azeem *et al.* 2013) and its unsuitability as food source (Heděnec *et al.* 2013).

5.3 CONCLUSION AND OUTLOOK

Overall, the results of the present PhD project provide further evidence for the importance of fungal chemical properties, fungal volatiles in particular, in affecting the outcome of fungus-arthropod interactions, as it has already been suggested by earlier studies (Böllmann *et al.* 2010, Rohlfs 2015, Rohlfs *et al.* 2007, Rohlfs and Churchill 2011, Stötefeld *et al.* 2012). I could clearly demonstrate that two important decomposers - Collembola and isopods - use fungal-derived volatiles as cues to make adaptive foraging decisions. In the light of the finding that Collembola are able to discriminate between fungi of varying suitability by detecting and responding to characteristics in fungal volatile profiles, it can be assumed that the use of volatile cues is an important, hitherto underestimated and underexplored, aspect of the food-selection process of Collembola and represents a mechanism that leads to fitness optimisation. Whether the emission of certain volatiles by filamentous fungi actually functions as defense needs to be tested by directly linking volatile-mediated responses of animals to the fitness of fungi, which can be best achieved for example by the use of genetically manipulated fungi deficient in the production of suspected deterrent volatiles.

Research on the function of fungal volatiles as infochemicals in fungus-invertebrate interactions is challenged by the high diversity of fungal-derived volatiles (Chiron and Micherlot 2005), synergistic effects between volatile compounds, as well as the high species-, genotype-, growth stage-, and physical state-related variability of fungal volatile emission and their dependency on the growth substrate (e.g. Börjesson *et al.* 1992, Combet *et al.* 2009, Fiedler *et al.* 2001, Gallois *et al.* 1990, Splivallo *et al.* 2012, Sunesson *et al.* 1995) (Chapter 4), and synergistic effects. It becomes even more complicated in the light of the world's vast diversity of fungal species with estimates between 500 thousand to 9.9 million (Hawksworth 2001) and their cryptic lifestyle. Future studies need to consider and incorporate this variation. Especially the importance of oxylipin volatiles as infochemicals in soil fungus-arthropod interactions needs to be investigated in further detail to find out whether fungal-derived oxylipins are of

similar importance for soil living arthropods as plant-derived oxylipins for herbivorous insects. Future studies need to include further species of fungivorous arthropods and should consider single but also synergistic and/or additive effects of different fungal volatiles e.g. by means of additive and subtractive methods. In the present study I could demonstrate that a video-based direct observation approach of the foraging responses of arthropods during both the searching (off-patch) and contact phase (on-patch) is a suitable method for gaining more detailed information on the overall food selection process.

Considering the opaque nature and complexity of soil habitats observations in natural environments are complicated. The advantage of laboratory experiments is the possibility to manipulate and control influencing variables, which facilitates conclusions and predictions of simple cause and effect relationships; nevertheless, carrying out experiments in more natural environments is crucial for the validation of effects in natural habitats. Investigation of fungus-arthropod interactions in semi-natural environments by means of microcosms experiments has been shown to be an appropriate approach to gain insights into complex interrelationships between organisms and environmental factors. Furthermore, an increasing number of studies show that the microcosm approach allows to measure ecosystem relevant impacts of organismic interactions, e.g. population dynamics, food-web structures, decomposition rates, nutrient distribution and -cycling (A'Bear *et al.* 2014b).

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THESIS DECLARATIONS

DECLARATION OF THE AUTHOR'S OWN CONTRIBUTION TO MANUSCRIPTS WITH MULTIPLE AUTHORS

In all manuscripts I am the first author and, with assistance of my supervisor Prof. Dr. Marko Rohlfs, I developed the main ideas and the study design.

Chapter 2: The 'substrate biting' experiment was conducted by Sandra Granzow during the course of her MSc thesis, the 'on-patch' experiment was conducted by Dominique Treschnak during the course of his BSc thesis, and I conducted the 'off-patch' experiment and the GC-MS volatile profiling. I analysed all data, created tables, figures and appendices, and wrote the manuscript.

Chapter 3 and Chapter 4: I collected and analysed the data, created tables, figures and appendices, and wrote the manuscript.

PLAGIARISM DECLARATION

I declare that I have written this doctoral thesis independently. All persons contributing to the manuscripts have been named so. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted this thesis in any form for another degree at any university or institution.

Laura Stötefeld
Göttingen, October 2018